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Biochemical and Genetic Characteristics of Bacteriocins of Food-Associated Lactic Acid Bacteria

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ABSTRACT

Numerous strains of lactic acid bacteria associated with food systems are capable of producing bacteriocins, or antibacterial proteins with activity against foodborne pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, and *Clostridium botulinum*. Recently, considerable emphasis has been placed on the identification of these proteins and their biochemical properties, including spectrum of activity, production conditions, purification procedures, amino acid composition, amino acid sequence, and modes of action. Advances in genetic engineering techniques have facilitated the characterization of these proteins at the genetic level, providing information on hydropathic index, protein synthesis and structure, immunity determinants for resistance and/or tolerance, and cloning of bacteriocin genes into other organisms. Prior to the utilization of bacteriocins and/or the producer organism in food systems, a thorough understanding of the physical, biochemical, and genetic properties is required. Additional parameters for use of bacteriocins and bacteriocin-producing organisms in food systems also are discussed.

Lactic acid bacteria are industrially important organisms recognized for their fermentative ability as well as their health and nutritional benefits (*20,49,85,120,121,129,130*). Species used for food fermentations belong to the genera *Lactococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Lactobacillus*, and the newly recognized *Carnobacterium* (*85*). These organisms have been isolated from grains, green plants, dairy and meat products, fermenting vegetables, and the mucosal surfaces of animals (*85*). Once used to retard spoilage and preserve foods through natural fermentations, they have found commercial applications as starter cultures in the dairy, baking, meat, vegetable, and alcoholic beverage industries. They produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins or bactericidal proteins during lactic fermentations (*85*). Not only are these components desirable for their effects on food flavor and texture, but they also inhibit undesirable microflora. Hence, lactic acid bacteria and their products give fermented foods distinctive flavors, textures, and aromas while preventing spoilage, extending shelf life, and inhibiting pathogenic organisms.

[1]*Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.*

BACTERIOCINS

Bacteriocins are proteinaceous compounds produced by bacteria that exhibit a bactericidal or bacteriostatic mode of action against sensitive bacterial species (*76,140*). Lactic acid bacteria have been studied extensively for bacteriocinogenicity. Considerable emphasis has been placed on the identification of bacteriocins from lactic acid bacteria associated with dairy products. Researchers presently are isolating strains of lactic acid bacteria associated with meat and vegetable fermentations and characterizing their bacteriocins. Methods for detection, characterization, purification, and identification of genetic determinants of bacteriocins from gram-positive (*140*) and gram-negative organisms are addressed in several reviews (*15,57,77,92,117*) and encyclopedia articles (*5,67*). Additional reviews provide overviews of bacteriocins from lactic acid bacteria (*23,47,76,107,123*). In this review, only those lactic acid bacteria isolated from food systems and their bacteriocins will be discussed. Each section is comprised of a genus of lactic acid bacteria and several known bacteriocins. In addition to the sources of the producing organisms, the most recent biochemical information (proteolytic susceptibility or degradation, activity spectrum, production conditions, purification procedures, isoelectric point, pH stability, amino acid sequence or composition analysis, molecular mass determination, or mode of action), and genetic data (localization of production gene, sequence analysis, cloning parameters, synthesis, structure, or immunity determinants) for each known bacteriocin are provided when available. Applications of bacteriocins and/or bacteriocin-producing organisms in food systems will be facilitated by a thorough comprehension of biochemical and genetic data as well as the inherent characteristics of the foods in which they are used.

BACTERIOCINS OF LACTOCOCCI

Lactococci are widely used as starter cultures in the dairy industry. Several strains of dairy species produce bacteriocins (Table 1). *Lactococcus lactis* and subspecies produce diplococcin, lactococcin, lactostrepcins, or nisin. Diplococcin and lactostrepcins are small molecular weight proteins active towards other lactococci. Nisin is a lantibiotic with inhibitory activity towards several gram-positive organisms. Lantibiotics differ from other lactococcal bacteriocins in their structure (unusual amino acids with sulfide bridging: aminobutyric acid, dehydroalanine, β-methyldehydroalanine, β-methyllanthionine) and synthesis (post-translationally modified to form dehydro-amino acids). More biochemical and genetic information is available about bacteriocins from lactococci than from any other genus grouped within the lactic acid bacteria.

TABLE 1. *Characteristics of bacteriocins of lactococci.*

Bacteriocin	Producer	Molecular mass (daltons)[a]	Stability	Sensitivity	Production parameters	Inhibitory spectrum	Gene locus	Mode of action	Reference
Diplococcin	*Lactococcus lactis* subsp. *cremoris* 346	5,300 (amino acid analysis)	-75°C; SDS	Chymotrypsin, trypsin, pronase, pepsin; temperature >4°C	Produced in milk, M17 broth; early stationary growth	*L. lactis* subsp. *lactis*, subsp. *cremoris*	83 kb conjugative plasmid	Cessation of DNA, RNA synthesis, decreased protein synthesis	*27-31,104*
Lactostrepcins	Non-nisin producing strains of *Lactococcus lactis* subsp. *lactis*, *cremoris*, *diacetylactis*	>10,000	Heat (121°C, 10 min); pH<5.0	Trypsin, pronase, chymotrypsin, lipases; pH>7.0	Produced in milk, rich complex broth, static broth cultures; early log growth	Lactococci; Group A, C, and G streptococci; *B. cereus*, *Lb. helveticus* *Lb. citrovorum* *Lb. paracitrovorum*	Not determined	Not determined	*38,80 152,153*
Lactostrepcin 5	*Lactococcus lactis* subsp. *cremoris* 202	>20,000 (SDS-PAGE)	Heat (121°C, 10 min); pH<5.0	Trypsin, pronase, lipase A	Static broth cultures; early log growth	Lactococci	Not determined	Ion leakage; interference with uridine transport; inhibition of DNA, RNA, and protein synthesis	*38,80,152*
Lactococcin I	*L. lactis* subsp. *cremoris* AC1	6,000	SDS, heat (100°C, 30 min); pH 4.5-7.0	Proteolytic enzymes	Milk, lactic broth; log growth	Lactococci, clostridia	60 kb conjugative plasmid	Not determined	*48*
Lactococcin A	*Lactococcus lactis* subsp. *diacetylactis* WM4, *L. lactis* subsp. *cremoris* LMG2130, 9B4	3,400 (genetic data)	20°C in 60% ethanol; pH 7.3 in 2.5 mM sodium phosphate; heat (100°C, 30 min); chymotrypsin	Endoprotease glu-C, trypsin	Produced in milk, M17 broth; log-early stationary growth	*L. lactis* subsp. *cremoris*, subsp. *diacetylactis*, clostridia	55 kb plasmid (1.2 kb fragment); 60 kb conjugative plasmid (18.4 kb fragment); 131.1 kb conjugative plasmid (9.5 and 13.4 kb fragments)	Leakage of intracellular components	*58,66,100 105,122 137,145,146*
Lactococcins M and N	*L. lactis* subsp. *cremoris* 9B4	69 amino acids (genetic data)	Not determined	Not determined	Not determined	Not determined	60 kb plasmid (1.8 kb fragment)	Not determined	*146,147*
Lactococcin B	*L. lactis* subsp. *cremoris* 9B4	5,300	Not determined	Not determined	Not determined	Not determined	60 kb plasmid (1.2 kb fragment)	Not determined	*147*
Nisin	Various strains of *L. lactis* subsp. *lactis*	3,500 (monomer; genetic data)	Heat (100°C, 10 min); pronase, trypsin, pepsin under acidic conditions	Chymotrypsin	Produced in milk, buffered, complex medium; log growth	Lactococci, bacilli, clostridia, *S. aureus*, micrococci	Plasmid or genomic DNA (8.5 kb fragment)	Efflux of amino acids and cations; collapse of membrane potential	*16,19,36,39 40,45,46,50 53-55,61 69,70,74,78 79,86,87,116 119,132,142*
Lacticin 481	*L. lactis* subsp. *lactis* CNRZ 481	Estimated 1,300-2,700	Heat (100°C, 1 hr)	Proteolytic enzymes	EGP broth, pH 5.5, early stationary growth	Lactococci, lactobacilli, leuconostocs *Cl. tyrobutyricum*	Plasmid	Not determined	*106,108*

[a] Method of molecular weight determination, if known, is included.

Diplococcin

One of the earliest bacteriocins identified in lactic acid bacteria, diplococcin is a 5300 Da protein produced by *L. lactis* subsp. *cremoris* strain 346 (*30,104*) in milk and M17 broth cultures during early stationary phase. It has been purified by chromatographic techniques (*30,31*), is unstable at room temperature, rapidly inactivated by heat, and degraded by the proteolytic enzymes chymotrypsin, trypsin, and pronase. Amino acid composition analysis showed a high content of acidic and neutral acids and a low content of basic amino acids (*31*). Diplococcin inhibits DNA and RNA synthesis in sensitive cells of selected strains of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* resulting in death without lysis (*27*). Production of and immunity to diplococcin are associated with an 83 kb conjugative plasmid (*28,29*). Additional genetic information about diplococcin is not available.

Lactostrepcins

Lactostrepcins are a group of acid tolerant bacteriocins produced by non-nisin producing strains of *L. lactis* biovar *diacetylactis*, some strains of *L. lactis* subsp. *cremoris*, and most strains of *L. lactis* subsp. *diacetylactis* (*38,80,152,153*). Full activity of these compounds occurs at acidic pH (<5.0), whereas loss of activity is demonstrated at pH 7.0 (*80*). Lactostrepcins are inactivated by proteolytic enzymes, are stable at 121°C for 10 min, and are produced in nonagitated broth cultures during early logarithmic growth (*38,80*). Based on dialysis experiments and ultracentrifugation, the molecular masses of these bacteriocins were found to exceed 10,000 Da (*38*). Whether lactostrepcins associate with cell fragments or medium components, like many other lactic acid bacteriocins, has not been determined. Further purification is required for definitive assessments of size. Antagonistic activity has been detected against other lactococci, group A, C, and G streptococci, *Bacillus cereus*, *Lactobacillus helveticus*, *Leuconostoc mesenteroides* subsp. *cremoris*, and *Leuconostoc paracitrovorum* (*80*). Lactostrepcin 5 produced by *L. lactis* subsp. *cremoris* 202 disrupts the integrity of the cell membrane resulting in ion leakage. Lactostrepcin 5 also interferes with uridine transport and inhibits DNA, RNA, or protein synthesis (*152*). Information regarding the genetic determinants responsible for production and immunity is inconclusive. Plasmid analysis of some producer strains and subsequent curing experiments did not indicate that plasmid DNA was responsible for bacteriocin production (*80,152*). The inability to link bacteriocin production with plasmid DNA suggests the possibility of genomic regulation of some of these proteins. For other strains, bacteriocin and immunity genes may not be in close proximity to one another (i e., one gene on chromosome, the other on a plasmid). In the case of the latter, plasmid curing studies may eliminate one set of genes, thereby affecting production and subsequent detection of the bacteriocin.

Lactococcin I

Lactococcin I has not been as thoroughly characterized as other bacteriocins from *L. lactis* subsp. *cremoris*. Lactococcin I is produced by *L. lactis* subsp. *cremoris* strain AC1 and inhibits other lactococci and some clostridia. The crude bacteriocin exists as a large, nondialyzable, carbohydrate-protein aggregate and is dissociated by sodium dodecyl sulfate (SDS) (*48*). Purified lactococcin I is a heat stable (99°C, 30 min) peptide with a molecular mass of 6000 Da. An 18.4 kb fragment of DNA from a 60 kb conjugative plasmid encodes lactococcin I production (*48*). Further characterization of the bacteriocin has not been reported.

Lactococcin A

Lactococcin A has been identified and characterized biochemically (*66*). Lactococcin A is produced by *L. lactis* subsp. *cremoris* LMG2130 constitutively during growth in M17 broth (*66*) or various other media (*105*). Purification from culture supernatants of M17 broth involved precipitation with ammonium sulfate (28% saturation), cation exchange chromatography, followed by reverse phase chromatography (*66*). Lactococcin A is bactericidal to *L. lactis* subsp. *lactis*, is sensitive to proteolytic enzymes, has an isoelectric point of 9.2, is insoluble in water, and is stable in a solution containing 60% ethanol and 2.5 mM sodium phosphate (pH 7.3) at -20°C (*66*). The bacteriocin is rich in alanine and glycine residues as determined by amino acid analysis. Sequence analysis of the purified protein identified 54 amino acid residues with a calculated molecular mass of 5778 Da (*66*). Lactococcin A apparently affects the cytoplasmic membrane of sensitive cells since leakage of intracellular components was detected (*66*).

Production of lactococcin A by *L. lactis* subsp. *cremoris* LMG2130 is associated with a 55 kb plasmid (*66*). Three fragments within the plasmid (0.6, 1.2, and 4.0 kb) hybridized with DNA probes deduced from the amino acid sequence and confirm the presence of the lactococcin A structural gene (*66*). The nucleotide sequence of the 1.2 kb fragment contains the lactococcin A structural gene and two open reading frames (ORFs). A ribosomal binding site and an ORF are located upstream from the structural gene. Within the ORF are three promoter regions which might initiate transcription of the protein (*66*). The lactococcin A structural gene encodes a precursor protein consisting of 75 amino acid residues. Twenty-one residues are part of an N-terminal extension with the remaining 54 amino acids constituting the active bacteriocin. Downstream from the structural gene is a ribosomal binding site followed by ORF2. ORF2 appears to encode a 98 amino acid polypeptide that consists of a 15 amino acid N-terminus and an active product of 79 to 88 amino acids. This particular protein, whether secreted or anchored within the membrane, may be responsible for bacteriocin immunity or resistance (*66*). Two high copy number plasmids, pON2 and pON7, were constructed from the 1.2 kb and 4.0 kb fragments. When the plasmids were transformed into *Escherichia coli*, no active bacteriocin was detected (*66*). *L. lactis* subsp. *cremoris*, transformed with pON2 or pON7, produced no detectable bacteriocin although it exhibited resistance. Another dairy strain, *L. lactis* subsp. *lactis* IL1403, transformed with pON2 and pON7, produced bacteriocin (60 bacteriocin units, BU/ml), albeit considerably less than the parental strain (1,500 BU/ml). This research provided evidence that transcription of lactococcin A occurs in *L. lactis* and that additional factors may be necessary for production of a functional bacteriocin (*66*) in *L. lactis* subsp. *cremoris*.

In addition to *L. lactis* subsp. *cremoris* LMG2130, other strains of *L. lactis* subsp. *cremoris* also produce lactococcin A. A bacteriocin-producing strain of *L. lactis* subsp. *cremoris*, strain 9B4, has been identified (*100*). Production of an antagonistic compound was associated with a 60 kb plasmid, p9B4-6, that contained at least two regions (1.3 and 1.8 kb) responsible for bacteriocin production and immunity. Although about 70% of p9B4-6 was cloned into *E. coli*, neither extracellular nor intracellular bacteriocin activity was detected (*145*). Transfer of the cloned plasmid DNA from *E. coli* into *L. lactis* subsp. *lactis* IL1403 resulted in the identification of two bacteriocins. One bacteriocin demonstrated considerable inhibitory activity towards *L. lactis* subsp. *lactis* Bu2-60 and Bu2-61, while the other showed less activity and was active against only Bu2-60 (*145*).

Further cloning, sequencing, and analysis of the 1.3 and 1.8 kb regions indicate that each fragment contains several operons responsible for bacteriocin production (*145,146*). The 1.3 kb fragment from p9B4-6, specifying lactococcin A activity, has been cloned and sequenced (*145,146*). The resulting plasmid, pMB553, contains two ORFs associated with bacteriocin production and immunity. ORF-B1 encodes a protein with an approximate molecular mass of 8.1 kDa (*146*). However, the active bacteriocin has a molecular mass of about 3.4 kDa, suggesting atypical migration of the bacteriocin in SDS-PAGE or post-translational processing of the 8.1 kDa-protein (*146*). The second reading frame (ORF-B2)

encodes a polypeptide consisting of 98 amino acids, possibly an immunity protein. Both ORFs are preceded by putative ribosomal binding sites (*146*). Further studies have shown that the nucleotide sequence of lactococcin A obtained from Holo et al. (*66*) is identical to the sequence of ORFB-1 of pMB553 (*146*). Amino acid sequence information of this fragment also indicates that the N-terminal extension of the polypeptide is cleaved off to produce the mature bacteriocin molecule (*146*). In addition to homology between other nucleotide sequences of lactococcin A (*66*) the genes for lactococcin A production and immunity are located on a plasmid similar in size (55-60 kb) to p9B4, but restriction enzyme profiles of the plasmids differ (*66*).

Lactococcus lactis subsp. *lactis* biovar *diacetylactis* WM4 also produces lactococcin A (*58,122,137*). As initially identified from the producer organism, the bacteriocin was active against *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, and *L. lactis* subsp. *diacetylactis* (*122,137*). Enzymatic treatment with most proteolytic enzymes inactivates the protein, but treatment with α-chymotrypsin has no effect (*122,137*). A 131.1 kb conjugative plasmid, pNP2, encodes bacteriocin production in this organism (*58,122,137*). Restriction enzyme analysis demonstrated that two contiguous restriction fragments, 9.5 and 13.4 kb from pNP2, encode bacteriocin production and immunity (*58*). Upon further subcloning, a 5.5 kb piece from the 9.5 kb fragment was found to be responsible for bacteriocin production (*137*). Nucleotide sequence analysis revealed five complete ORFs within the 5.5 kb fragment (*137*). The nucleotide sequences of ORF1 and ORF2 code for proteins that may be associated with bacteriocin secretion in producer organisms (*137*). Nucleotide sequence information indicated that ORF3 is involved with bacteriocin production and ORF4 was responsible for immunity determinants. Known nucleotide sequences of lactococcin A (*66,146*) were 100% homologous to ORF3 and ORF4 (*137*) and indicate that the bacteriocin of *L. lactis* subsp. *lactis* biovar *diacetylactis* WM4 is lactococcin A. ORFX, the fifth ORF of this cluster, has no function in bacteriocin production or immunity (*137*). It was concluded that production and complete secretion of active lactococcin A is dependent upon the presence of all four ORFs (*137*). The homologous nucleotide sequences of the genetic and immunity determinants of lactococcin A from three different strains of *Lactococcus* sp. are intriguing and may suggest some evolutionary event or the possibility of transposition-like events between lactococci (*66*).

Lactococcin M

The 1.8 kb region of p9B4-6, from *L. lactis* subsp. *cremoris* 9B4, associated with production of another bacteriocin, was also cloned and analyzed (*146*). Nucleotide sequence analysis of the resulting plasmid (pMB225) showed a ribosomal binding site followed by three ORFs, designated ORFA-1, ORFA-2, and ORFA-3 (*146*). Further analysis demonstrated that ORFA-1 and ORFA-2 encode 69 and 77 amino acid proteins, respectively (*146*). Both ORFs appear to participate in production of the bacteriocin, lactococcin M (*146*). While ORFA-1 may be responsible for production of the active protein, it is possible that the polypeptide encoded by ORFA-2 was responsible for maturation or secretion of lactococcin M (*146*). Recently, ORFA-1 and ORFA-2 have been renamed to *lcn* M and *lcn* N (*147*). The third ORF, ORFA-3 (*lci* M), appears to encode an immunity protein for lactococcin M (*146*).

Lactococcin B

Lactococcin B is a bacteriocin associated with a 1.2 kb fragment from the bacteriocinogenic plasmid p9B4-6 of *L. lactis* subsp. *cremoris* 9B4 (*147*). The genes encoding bacteriocin production are located on the same fragment as those of lactococcin M and lactococcin A (*147*). The nucleotide sequences of the structural genes for production and immunity of lactococcin B were determined following deletion and mutation analyses of the 1.2 kb fragment. Both genes were found within one operon (*147*). Part of the nucleotide sequence of this operon is homologous to DNA responsible for synthesis of leader sequences associated with the operons of lactococcin M and lactococcin A (*147*). Nucleotide sequence information also suggests that the bacteriocin is synthesized as a 7600 Da protein, followed by deletion of 21 N-terminal amino acids for a final active protein of 5300 Da (*147*). Subsequent cloning experiments involved the incorporation of the operon into an *E. coli* expression vector with a T7 RNA polymerase-specific promoter (*147*). Following induction of the T7 RNA polymerase gene with IPTG (isopropyl β-D-thiogalactopyranoside), cell-free supernatants were subjected to tricine SDS-PAGE and examined for bacteriocin activity (*147*). Lactococcin B activity by *E. coli* was observed in a protein band corresponding to 6500 Da. Size differences were observed between the bacteriocin present in SDS-PAGE and the calculated molecular mass from nucleotide sequence information. Discrepancies between the two size determinations are attributed to the hydrophobic nature of the protein which alters migration in SDS-PAGE (*147*). Additional information indicates that 21 of the N-terminal amino acids of lactococcin M, lactococcin B, and lactococcin A are nearly identical, suggesting that these bacteriocins are processed at the same position within p9B4-6 (*147*). Not only is the presence of genes for three bacteriocins within one plasmid unique but the partial homology of similar DNA regions indicates some amount of recombination (*147*). The organization of three bacteriocins within one plasmid provides *L. lactis* subsp. *cremoris* 9B4 with a selective advantage over other bacteriocin-producing organisms in the same environmental niche (*147*). This study also represents the first successful cloning and expression of a bacteriocin from lactic acid bacterium in a gram-negative host.

Nisin

Nisin is the most extensively characterized bacteriocin of the antimicrobial proteins produced by lactic acid bacteria (*19,39,40, 44-46,50,53-55,74,78,79,86,87,119,132,142*). Nisin also is the subject of many reviews (*36,55,61,69,70,86*). Nisin belongs to the lantibiotics, a family of peptides containing α, β-unsaturated amino acids (dehydroalanine and dehydrobutyrine) and thioether amino acids (lanthionine and β-methyllanthionine; *53,54*). Nisin is a bacteriocin with a molecular mass of 3500 Da (34 amino acid residues; *54*). It is active against most gram-positive bacteria including lactococci, bacilli, micrococci, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Clostridium botulinum* (*69,70*). It is heat stable (100°C for 10 min), inactivated by chymotrypsin, and resistant to pronase and trypsin under acidic conditions (*53,54*). Media for nisin production include milk and buffered, complex media. Production of nisin occurs during logarithmic growth. Conventional protein-synthesizing pathways involving transcription and translation are responsible for production of the nisin precursor, pronisin. Pronisin contains serine, cysteine, and threonine residues (*19,116*). It is transported to the cell surface during the exponential phase of growth and at the cell surface, is enzymatically converted to nisin (*19,116*). Serine and threonine are dehydrated to the corresponding dehydro amino acids which in turn condense with cysteine to form lanthionine and β-methyllanthionine (*116*). This conversion explains the synthesis of the unusual amino acids found in active nisin.

Nisin activity appears to be directed against the cytoplasmic membrane (*16,46,78,79,119*). Following nisin treatment, whole or intact sensitive cells and membrane vesicles exhibit efflux of amino acids and cations (*46,78,79,119*). Loss of these substances depletes proton motive force (*16*), which ultimately interferes with cellular biosynthesis (*119*). These events result in collapse of the membrane potential and ultimately cause cellular death (*46,119*).

Once thought to be active against only gram-positive bacteria, nisin has been used in conjunction with EDTA, to effectively reduce populations of *Salmonella* sp., *Shigella*, and *E. coli* (134-136). In these studies, 10^7 CFU/ml of *Salmonella* sp. were treated with a solution containing 50 μg/ml of nisin and 20 mM EDTA (pH 6.5, 37°C, 30 min and 1 h; *134-136*). Simultaneous treatment of cells with EDTA and nisin resulted in a 3.2 to 6.9 log reduction in the population of *Salmonella* sp. (*134-136*). Addition of EDTA to gram-negative cultures chelates divalent cations, thereby increasing cell permeability and susceptibility to antibiotics, detergents, and bacteriocins. Divalent cations, such as magnesium, are essential to the maintenance of the lipopolysaccharide (LPS) of the gram-negative outer membrane since they decrease the electrostatic repulsions, thereby excluding hydrophilic, hydrophobic, and amphiphilic molecules from the LPS (*135*). Alteration of the gram-negative outer membrane by removal of these cations is necessary before nisin can affect the cytoplasmic membrane.

Location of the nisin gene varies among strains of lactococci. Nisin production and immunity can be mediated by plasmid DNA in *L. lactis* subsp. *lactis* (*39,40,44,45,142*). However, some results indicate that production is mediated by either chromosomal DNA (*50,133*) or a conjugal transposon (*40*). Genetic determinants for nisin production and immunity have been transferred conjugally with expression of the active protein (*50,142*). However, production of active nisin from experimentally cloned DNA has not yet been demonstrated.

Genes for production of and immunity to nisin are located in an operon of at least 8.5 kb (*55*). The nucleotide sequences of genes encoding the peptide precursor, pronisin, and immunity determinants have been deduced (*19,39,45,55,74,132*). The nisin structural gene is part of a polycistronic operon (*133*) that probably includes a single promoter located upstream from the structural gene and is responsible for initiation of transcription. An ORF lacking a promoter was located downstream. The primary translation product from the sequence of the *nis* A structural gene is a 57-amino acid residue pronisin peptide. The 34-amino acid residue C-terminal portion appears in active nisin. The remaining 23 residue N-terminal portion is removed by enzymatic cleavage at the cell surface of *L. lactis* subsp. *lactis* (*19*). Transcription of pronisin is dependent upon read-through from the upstream gene. The structure of nisin, therefore, is dictated by the sequence of the structural gene, in contrast to other peptide antibiotics that are synthesized by nonribosomal pathways and involves multi-step enzymatic pathways (*55*).

Lacticin 481

L. lactis subsp. *lactis* CNRZ481 produces the lantibiotic lacticin 481 (*106,108*). It is active against other lactococci, lactobacilli, leuconostocs, and *Clostridium tyrobutyricum* (*106*). Maximum production of lacticin 481 occurs when cultures are maintained at a constant pH of 5.5 (*106*). Initial studies focused on the identification and characterization of the bacteriocin, followed by its production in cheese (*106*). Lacticin 481 is a proteinaceous, heat stable (100°C, 1 h) compound with molecular mass approximating 5,000 to 10,000 Da as determined by ultrafiltration and gel filtration chromatography (*106*). Inoculation of both producer and sensitive organisms into cheese milk resulted in production of the bacteriocin and inhibition of the sensitive organism (*106*).

In another study, conjugal matings of *L. lactis* subsp. *lactis* CNRZ 481 with *L. lactis* subsp. *lactis* IL1441 resulted in the isolation of a Bac^+, Bac^r transconjugant, *L. lactis* subsp. *lactis* T_H (*108*). Upon reaching stationary growth in Elliker broth containing 15 g/L sodium β-glycerolphosphate (EGP) medium at a constant pH of 5.5, the culture was adjusted to pH 6.0, filtered, treated (10 min, 100°C) to increase stability and stored at -20°C (*108*). Following 60 and 80% ammonium sulfate fractionation (455-fold specific activity increase), lacticin 481 was further purified by gel filtration chromatography and reversed phase high-performance liquid chromatography (HPLC) (*108*). SDS-PAGE analysis followed by electroblotting of the protein to PVDF membranes revealed a single protein band at 1,700 Da upon Coomassie blue staining. Inhibitory activity of the band towards a sensitive indicator organism was detected (*108*). Amino acid sequence analysis of lacticin 481 only identified seven residues from the amino terminus. The amino acid composition of the purified bacteriocin revealed 30 residues, including lanthionine (*108*). However, the amino acid composition of this protein was not similar to other lantibiotics (nisin, epidermin, subtilin, gallidermin, pep5). The presence of a dimer or trimer, hydrophobicity of the protein, and atypical migration of lantibiotics during SDS-PAGE may account for the discrepancies of molecular size that were encountered during purification (*108*). The calculated molecular masses of lacticin 481 were 2,400 to 2,700 Da following amino acid composition, 1,300 Da after gel filtration chromatography, and 1,700 Da on SDS-PAGE (*108*). More accurate assessments of the size of this protein await elucidation of the molecular organization of lacticin 481.

BACTERIOCINS OF PEDIOCOCCI

Pediococci are involved with the fermentation of many vegetables, cheeses, meats, and sausage products. Pediocins are bacteriocins produced by three species within the genus *Pediococcus: P. acidilactici, P. cerevisiae*, and *P. pentosaceus*. Pediocins exhibit a wide spectrum of activity against gram-positive organisms including lactic acid bacteria, *L. monocytogenes, S. aureus*, and clostridia (*8,11,24,42,51,71,89,109,118,123*). In general, pediocins have been less extensively characterized than nisin or other bacteriocins from lactic acid bacteria. The biological, chemical, and genetic characteristics of pediocins (Table 2) will be discussed in this section.

Pediocin AcH

Pediococcus acidilactici strain H was isolated from fermented sausage (*10*). The bacteriocin pediocin AcH is active against many lactobacilli, leuconostocs, *S. aureus, Clostridium perfringens, L. monocytogenes*, and *Pseudomonas putida*. Pediocin AcH is sensitive to proteolytic enzymes, stable at 121°C for 15 min, and stable at pH 2.5 to 9.0 (*11*). Production of the bacteriocin occurs during stationary growth in TGE (trypticase, glucose, yeast extract) broth at pH 6.5 (*14*). Pediocin AcH was purified by precipitation of cell-free culture supernates with ammonium sulfate (70%), dialysis against deionized water, resuspension in 6 M urea and ammonium acetate, gel filtration, and anion exchange chromatography (*11*). SDS-PAGE analysis of the crude sample revealed several protein bands ranging in molecular mass from 2,700 to 50,000 Da (*9,10*). When the SDS-PAGE gel was overlaid with soft agar containing a sensitive organism, inhibitory activity corresponded to a 2,700 Da band (*10,11*). Following purification, the protein band was transferred from the gel to a PVDF membrane and subjected to N-terminal sequence analysis in which 23 amino acids were identified: NH_2-Lys-Tyr-Tyr-Gly-Asn-Gly-Val-Thr-Cys-Gly-Lys-His-Ser-Cys-Ser-Val-Asp-Trp-Gly-Lys-Ala-Thr-Thr-. . . . (*95*). Subsequent genetic data revealed the presence of an 18 amino acid leader sequence, suggesting that the bacteriocin is translated as a prepediocin that undergoes post-translational processing to an active protein containing 44 amino acids (*95*). Further research will identify other post-translational modifications of pediocin AcH in which serines and threonines may be dehydrated and form thioester bonds with cysteines (*95*).

Pediocin AcH exhibits bactericidal activity against sensitive cells (*11*). The mode of action of this bacteriocin is thought to be related to inhibition of ATP synthesis, impairment of transport systems, or damage to permeability barriers of the cytoplasmic membrane (*13*). Pediocin AcH killed sensitive cells without causing cellular lysis or leakage of β-galactosidase. In a more comprehen-

TABLE 2. *Characteristics of bacteriocins of pedicocci.*

Bacteriocin	Producer	Molecular mass (daltons)	Stability	Sensitivity	Production parameters	Inhibitory spectrum	Gene locus	Mode of action	Reference
Pediocin AcH	*Pediococcus acidilactici* H	2,700 (SDS-PAGE)	Heat (121°C, 15 min); 6 M urea; pH 2.5-9.0	Trypsin, ficin, papain, proteinase K, chymotrypsin	Supplement TGE broth, pH 6.5; stationary growth	Lactobacilli, leuconostocs, *S. aureus*, *C. perfingens*, *L. monocytogenes*, *P. putida*	11.4 kb plasmid	Inhibition of ATP synthesis; impairment of transport systems	*9-14,95 113-115*
Pediocin PA-1	*Pediococcus acidilactici* PA 1.0	4,600 (nucleotide sequence)	Lipase, phospholipase, lysozyme, DNase, RNase; heat (80-100°C, 10 min); pH 4-7	Pepsin, papain chymotrypsin, protease	Broth; stationary growth	Pediococci, lactobacilli, *L. mesenteroides*, *L. monocytogenes*	9.4 kb plasmid	Not determined	*51,65,88 91*
Pediocin A	*Pediococcus pentosaceus* FBB61	Not determined	Heat (100°C, 60 min); -20°C	Pronase	On agar	Pediococci, lactobacilli *S. aureus*, *Cl. perfingens*, *Cl. botulinum*	21 kb plasmid	Not determined	*21,35,97*
Unnamed	*Pediococcus cerevesiae* FBB63	Not determined	Not determined	Not determined	Not determined	Pediococci lactobacilli leuconostocs	16 kb plasmid	Not determined	*35,46*
Unnamed	*Pediococcus acidilactici* PC	Not determined	Lipase, heat (100°C, 60 min), pH 4-8	Chymotrypsin, ficin, protease, trypsin	Broth cultures of MRS or semidefined media (pH 6.0)	*Cl. perfingens*, *Listeria* spp., leuconostocs pediococci	8.47 kb plasmid	Not determined	*51,68,71*

sive study, several parameters associated with the lethal action of pediocin AcH were discussed (*13*). Pediocin AcH adsorbed to gram-positive but not to gram-negative organisms. The lipotechoic acids (LTA) from sensitive cells were removed by organic solvents. Cells with LTA and without LTA were treated with pediocin AcH. Pediocin AcH bound only to the LTA and not to the cells, suggesting that LTA is a binding site. Sensitive cells leaked potassium ions and/or ultraviolet absorbing material, lost their ability to replicate, were more permeable to o-Nitrophenyl-β-D-galactospyranoside (ONPG), and in some cases, lysed (*13*). Binding to sensitive cells appeared to be dependent upon pH (6.5) and on the presence of anions. Treatment of sensitive cells with SDS, guanidine-HCl, several organic solvents, or enzymes did not affect binding (*13*). Addition of excess pediocin AcH to sensitive cells resulted in a saturation of receptor sites. Pediocin then attached at other sites and affected membrane integrity (*13*).

Bacteriocin production and resistance determinants in *P. acidilactici* strain H, the wild type strain, are linked to an 11.4 kb plasmid (*113*). Recently, *P. acidilactici* LB42-923, a transconjugant of *P. acidilactici* H, was found to harbor an 8.9 kb plasmid (pSMB74) responsible for pediocin AcH production (*113*). Restriction enzyme analyses of pSMB74 demonstrated that a 3.5 kb fragment (pRBI) was responsible for pediocin AcH production (*113*). Further analysis of pRBI localized pediocin AcH production to a 33 kb fragment. Two putative ribosomal binding sites, a start codon, a stop codon, and an ORF were identified within this fragment (*95*). The 23 amino acid N-terminal sequence of the purified bacteriocin demonstrated homology to the amino acids encoded by the fragment (*95*). Additional information pertaining to the immunity determinants for pediocin AcH is forthcoming.

The immunogenicity of pediocin AcH was evaluated in mice and rabbits (*12*). Studies indicated that these animals did not demonstrate antibody response upon intraperitoneal and subcutaneous injection of the bacteriocin, apparently because of the size of the pediocin AcH peptide (*12*). A sausage prepared using *P. acidilactici* H as a starter culture did not cause any apparent ill effect to the consumers over a period of time, presumably due to the inactivation of the bacteriocin by chymotrypsin and trypsin found in the gastrointestinal tract (*12*). This information implies that pediocin AcH may be consumed without toxic effects and, therefore, may be suitable for applications in foods (*12*).

Pediocin PA-1

P. acidilactici strain PAC-1.0 produces the bacteriocin, pediocin PA-1 (*51*). The bacteriocin is produced during stationary growth. Pediocin PA-1 inhibits other pediococci, lactobacilli, *Leuconostoc mesenteroides*, and *L. monocytogenes* (*109*). Pediocin PA-1 was partially purified from broth cultures by ammonium sulfate precipitation, dialyzed, and subjected to ion exchange chromatography (*51*). Partially purified pediocin PA-1 was sensitive to proteolytic enzymes but was not affected by lipase, phospholipase, lysozyme, DNase, or RNase. Bacteriocin activity was stable at 80 to 100°C and between pH 4 and 7. Gel filtration experiments identified a molecular mass of 16,500 Da for pediocin PA-1, a size comparable to bacteriocins from other lactic acid bacteria (*51*). Bacteriocin production was associated with a 9.4 kb plasmid. Confirmative evidence showed that loss of the plasmid during curing studies resulted in simultaneous loss of bacteriocin production (*51*). Adsorption studies were performed with partially purified bacteriocin and showed that pediocin PA-1 was bactericidal to sensitive cells. Nonlethal binding was observed since both sensitive and insensitive cells adsorbed the bacteriocin at comparable levels (*51*).

Pediocin PA-1 has been purified to homogeneity and the amino acid sequence determined in two separate studies (*65,88*). An 18-h culture of MRSYE (MRS broth with 2.5% yeast extract)

broth containing *P. acidilactici* strain PAC-1.0 was filtered, neutralized to pH 6.0, and subjected to gel filtration and ion exchange chromatography (*65*). Since pediocin PA-1 activity was observed in several eluents, active fractions were pooled, dialyzed, and concentrated by vacuum centrifugation (*65*). The concentrated sample was further purified by HPLC, resulting in a 470-fold purification step. SDS-PAGE analysis of the HPLC purified protein indicated a molecular mass of 8,600 Da for pediocin PA-1. Further analysis for amino acid structure and composition showed that pediocin PA-1 is a 44-residue polypeptide composed of aromatic and aliphatic amino acids. A predicted isoelectric point (pI) of the protein is 8.65 (*88*) and 10.0 (*65*), as deduced by sequence analysis. Sequence information predicted a molecular mass of 4,629 Da and differs from the 8,600 Da observed during SDS-PAGE analysis. The discrepancies in size determinations are attributed to abnormal migration of the small peptide in the gel matrix (*65*).

A recent study (*91*) represents the first successful cloning of structural and immunity genes as well as expression of a pediocin in *E. coli*. Initially, a 9.4 kb bacteriocinogenic plasmid, pSRQ11, was analyzed by deletion analysis and a 5.6 kb fragment was identified that was responsible for pediocin PA-1 production. Nucleotide sequence analysis of the fragment demonstrated a cluster of four ORFs, designated *ped* A, *ped* B, *ped* C, and *ped* D (*91*). A single putative promoter is located upstream from the cluster and suggests that the four genes are organized in an operon-like structure. Additional information showed that a 62 amino acid precursor was associated with *ped* A. Residues 19 to 62 of the gene corresponded to the amino acid sequence of the purified protein. These data show that pediocin PA-1 is a 44 amino acid protein with a molecular mass of 4,600 Da and that an 18 amino acid N-terminal peptide is removed prior to production of the active protein. Frameshift mutations within *ped* A resulted in loss of pediocin PA-1 activity and confirmed that alterations in the structural gene affect production of the active protein. *ped* B and *ped* C, located downstream from *ped* A, also encode proteins that are not associated with pediocin PA-1 production (*91*). Specifically, *ped* B encodes a 112 amino acid protein that may be involved with immunity. Plasmid curing studies also demonstrated that immunity determinants for the producer organism, *P. acidilactici* PAC1.0, may be located on the chromosome (*91*).

Another operon of the cluster, *ped* D, encodes a 724 amino acid peptide that is necessary for production of the bacteriocin in *E. coli* (*91*). The predicted protein encoded by this operon contains a hydrophobic N-terminus and an hydrophilic C-terminus. Sequence analysis of this protein and searches for homologous sequences in protein data bases indicate that the protein of *ped* D is closely related to other ATP-binding proteins (*91*). The significance of this information may be that the *ped* D protein is responsible for translocation, transport, and/or secretion of the protein in *E. coli* (*91*).

At present, pediocin PA-1 activity against *L. monocytogenes* has been observed in several food systems such as dairy products, fermented semi-dry sausage, and fresh meat. These studies also suggest the usefulness of cloning of the pediocin PA-1 genes into resistant lactic acid bacteria for the improvement of starter cultures used in various food systems (*91*).

Other pediocins

Several additional reports address bacteriocins produced by pediococci. Two strains of *P. pentosaceus* were isolated initially from cucumber fermentations (*41*). These organisms produce bacteriocins against a variety of gram-positive bacteria: *Pediococcus* sp., *Clostridium* spp., *S. aureus*, *Lactobacillus* spp. (*25*), *Bacillus cereus*, *Cl. perfringens*, and *Listeria* sp. (*131*). Activity was not detected against gram-negative bacteria, yeasts, or molds (*41*). One isolate, *P. pentosaceus* strain FBB61, produces the bacteriocin pediocin A. The bacteriocin is produced in agar cultures, activity is retained during dialysis, and it is resistant to heating (100°C, 60 min) and freezing (*118*). Attempts to purify and further characterize the bacteriocin were unsuccessful since production in broth was not detected. *P. pentosaceus* FBB61 harbors a 21 kb plasmid (pMD136; *25*). Plasmid curing experiments suggested that pediocin A production and immunity genes are plasmid-encoded (*25*); however, additional data pertaining to genetic regulation of pediocin A production are not available.

Additional reports link bacteriocin production in the pediococci to plasmid DNA. A bacteriocin-like compound was linked to *P. cerevesiae* strain FBB63, an organism isolated from a cucumber fermentation (*41*). The compound was active against several genera of lactic acid bacteria and exhibited similar activity to pediocin A. *P. cerevesiae* FBB63 harbors four plasmids (*52*). Curing experiments have linked a 16 kb plasmid to bacteriocin production. It was not determined if immunity determinants were associated with this plasmid.

Three strains of *P. acidilactici* and a strain of *P. pentosaceus* isolated from fermented sausage had similar plasmid profiles. All harbor an 8.47 kb plasmid (*68*). Plasmid profiles of the four strains were similar. The plasmid encoded production of a bacteriocin that was active against *L. monocytogenes*, *Enterococcus faecalis*, and *Leuconostoc mesenteroides* (*68*). No other additional data were reported for these bacteriocins.

Recently, a study reported that *P. acidilactici* PC produces a bactericidal protein that inhibits *Cl. perfingens*, *Listeria* spp., leuconostocs, and other pediococci (*71*). Produced in MRS or semi-defined medium (pH 6.0), the crude bacteriocin was sensitive to chymotrypsin, ficin, protease, trypsin, but not to lipase. Crude preparations of the bacteriocin were stable at 100°C, 60 min and between pH 4.0 and 8.0. Plasmid curing studies of *P. acidilactici* PC demonstrated that an 8.47 kb plasmid was responsible for production of the bacteriocin in this organism. Additional studies verified that plasmid profiles were identical to *P. acidilactici* PAC-1.0, which produces the bacteriocin pediocin PA-1 (*51*) and *P. acidilactici* PO2 described above (*68*). Genetic analysis of total genomic DNA by DNA fingerprinting and rRNA typing confirmed that *P. acidilactici* PAC-1.0, *P. acidilactici* PC, and *P. acidilactici* PO2 were identical organisms (*71*).

The identification and isolation of plasmid-borne traits, such as bacteriocin production, simplifies characterization of the genes for production and immunity. The use of DNA fingerprinting and rRNA typing should prevent duplication of bacteriocin production among strains. In addition, the ability to exchange such traits (either through conjugation or transformation) is possible (*75,114,115*). Presently, research is under way to determine the modes of action of several of the pediocins on sensitive cells as well as the use of the bacteriocin producer organisms and/or their compounds in food systems (*8,32,33,42,109*).

BACTERIOCINS OF LEUCONOSTOCS

Leuconostoc spp. are lactic acid bacteria found in raw foodstuffs, dairy products, and wine fermentations. Antimicrobial activity of these organisms has long been known and recognized (*76*). Most often, the antagonistic compounds are not bacteriocins but acetate or diacetyl (*76*). Several reports have addressed bacteriocin-producing strains of leuconostocs. In one study, wine and dairy isolates of *Leuconostoc* spp. produced bacteriocin-like compounds active against selected strains of *L. lactis* subsp. *lactis* (*103*). Other studies (*56,81,82,84*) have identified bacteriocin-producing strains of *Leuconostoc* spp. but complete biological, chemical, and genetic information is lacking. The identification and characterization of four known bacteriocins from *Leuconostoc* spp. will be discussed (Table 3).

Mesenteroicin 5

Mesenteroicin 5, is produced by *Leuconostoc mesenteroides* UL5, isolated from Swiss-type cheese (*22*). Mesenteroicin 5 dem-

TABLE 3. *Characteristics of bacteriocins of leuconostocs.*

Bacteriocin	Producer	Molecular mass (daltons)	Stability	Sensitivity	Production parameters	Inhibitory spectrum	Gene locus	Mode of action	Reference
Mesenterocin 5	*Leuconostoc mesenteroides*	4,500 (SDS-PAGE)	Heat (100°C, 30 min)	Pronase, chloroform	Early stationary growth	*L. monocytogenes*, *E. faecalis*, *B. linens*, *Pediococcus Pentosaceus*	Not determined	Bacteriostatic	*22*
Leucocin A	*Leuconostoc gelidum* UAL 187	3,900 (genetic data)	Lipase; pH 2-3; urea; chloroform; heat (62°C, 30 min)	Protease chymotrypsin, trypsin, papain, pepsin; pH>7.0	MRS broth, pH 6.0, early log growth	leuconostocs, lactobacilli, pediococci, *E. faecalis*, *L. monocytogenes*	11.7 kb plasmid (localized to 2.9 kb fragment)	Not determined	*2,4,56 62-64*
Leuconocin S	*Leuconostoc paramesenteroides* OX	2,000 (SDS-PAGE)	Heat (60°C, 30 min) 0.1% SDS	Amylase chymotrypsin trypsin pronase E proteinase K	APT broth, pH 6.15*	*Lb. sake* *L. monocytogenes*, *S. aureus*, *Y. enterocolitica* *A. hydrophila*	Not determined	Bacteriostatic, affects proton motive force	*81,84*
Carnocin	*Leuconostoc carnosum* LA44A	2,500-6,000 (SDS-PAGE)	Heat (100°C, 15 min); pH 2-10	Chymotrypsin, trypsin, amylase; heat 121°C, 15 min	modified MRS broth (pH 6.5), 18 h, 25°C or 148 h, 10°C	lactobacilli carnobacteria, enterococci, pediococci, leuconostocs, *Listeria* spp.	Not determined	Not determined	*148*

onstrates many of the properties of other bacteriocins of lactic acid bacteria. It is degraded by proteases, displays heat resistance (100°C for 30 min), is produced during early stationary phase of growth, and inhibits gram-positive bacteria such as *L. monocytogenes*, *S. faecalis*, *Brevibacterium linens*, and *P. pentosaceus* (*22*). The bacteriocin is precipitated by ammonium sulfate (60%), is retained in dialysis tubing, and can be concentrated by ultrafiltration. Mesenteroicin 5 also exhibits a bacteriostatic action against sensitive cells. SDS-PAGE analysis of the concentrated crude preparation revealed a smear, rather than a specific band, indicating the presence of contaminating proteins. Bacteriocin activity was detected in gels at an area corresponding to 4,500 Da (*22*).

To obtain bacteriocin-negative mutants, the bacteriocin producer organism, *L. mesenteroides* UL5, was subjected to three consecutive culturings in acriflavin (15 μg/ml). Analysis and comparison of one mutant to the parent strain indicated that it did not differ from the parent strain in acid formation or carbohydrate fermentations and was not inhibited by mesenteroicin 5 (*22*). Because immunity was transferred independently of bacteriocin production, it was concluded that the genes for bacteriocin production and immunity were not genetically linked (*22*). Genetic determinants for bacteriocin production were not discussed but will no doubt be addressed in upcoming reports.

Leucocin A

A strain of *Leuconostoc gelidum*, isolated from meat that was packaged under 30% carbon dioxide (*2,56,64*), produces antimicrobial activity that was unrelated to acid or hydrogen peroxide. Further characterization identified the bacteriocin leucocin A (*64*). Leucocin A is stable at low pH (2-3), is heat resistant (62°C for 30 min), and is inactivated by several proteolytic enzymes (protease, chymotrypsin, trypsin, papain, and pepsin). It inhibits leuconostocs, lactobacilli, pediococci, *E. faecalis*, and *L. monocytogenes* (*64*). Leucocin A is produced maximally during early log phase in broth cultures (pH 6.0, 37°C; *64*). Leucocin A was purified by ammonium sulfate precipitation (pH 2.5), passage through Amberlite XAD-2 and Sephadex G-25 columns, and HPLC analysis (*63*). SDS-PAGE analysis of the HPLC purified sample identified a protein band at 2,500 to 3,000 Da, whereas mass spectrometry identified a protein with a molecular mass of 3,930. Preliminary genetic studies linked production of leucocin A to an 11.7 kb plasmid (*64*). Amino acid analysis of the purified protein identified 37 amino acid residues (*63*). The N-terminal amino acid sequence consisting of 13 amino acids was determined. A 24-mer oligonucleotide probe was constructed from the amino terminal sequence and used to probe the plasmid containing structural genes for leucocin A. Both southern and colony blot hybridizations confirmed the presence of the leucocin A structural and immunity genes (*63*). The leucocin A gene is located within a 2.9 kb insert on the plasmid. Sequence analysis of the insert revealed 2 ORFs flanked by a putative promoter and terminator (*63*). Transcription of leucocin A begins at a promoter located upstream from the structural genes. The structural gene for leucocin A encodes a 61 amino acid prepeptide consisting of a 24 residue N-terminal extension and a 37 amino acid bacteriocin (*63*). The second ORF encodes for a protein consisting of 112 amino acids and may be an immunity protein (*62*). Subcloning of the 2.9 kb insert into the shuttle vector pNZ19 resulted in the hybrid plasmid, pJH8.6L. Electroporation of pJH8.6L into a bacteriocin-negative mutant of the parent strain did not allow for expression of leucocin A. Further research is under way to ensure expression of and immunity to the bacteriocin in nonproducer strains of lactic acid bacteria and *E. coli* (*4*).

Leuconocin S

L. paramesenteroides OX was isolated from retail lamb and found to produce the bacteriocin, leuconocin S (*81,84*). The bacteriocin is produced maximally during early stationary growth (within 12 h) at pH 6.15 in APT broth (*84*). Following production of the bacteriocin, experiments indicated that leuconocin S is a heat stable (60°C, 60 min) glycoprotein with activity against *L. monocytogenes*, *S. aureus*, *Lactobacillus sake*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, and some strains of *Cl. botulinum* (*81,84*). In addi-

tion to α-amylase (10 mg/ml, 30°C, 30 min), leuconocin S is inactivated by treatments with trypsin, chymotrypsin, protease, and proteinase K (*81,84*). The crude bacteriocin appears to affect the proton motive force of *Lb. sake*, resulting in a bacteriostatic effect on cells (*84*). SDS-PAGE analysis and alcian blue staining confirmed the glycoprotein nature of leuconcin S and estimated size of the bacteriocin. Bacteriocin activity migrated in two protein bands at approximately 10,000 Da and 2,000 Da (*84*). The presence of the 2,000 Da band may have been attributed to cleavage of the 10,000 Da protein (*84*). *L. paramesenteroides* OX harbors a 17 kb plasmid (*81*), but the association of this plasmid with bacteriocin production has not been determined. The broad spectrum of activity and the glyco nature of this protein are unique and warrant further research.

Carnocin

Leuconostoc carnosum LA44A was isolated from vacuum-packaged vienna-type sausages and found to produce the bacteriocin designated carnocin (*148*). Preliminary studies showed that carnocin was active towards lactobacilli, carnobacteria, enterococci, pediococci, leuconostocs, and *Listeria* spp. Crude extracts of the bacteriocin were inactivated by chymotrypsin, trypsin, an amylase, and heating at 121°C, 15 min but was stable between pH 2 and 10 and at 100°C for 15 min (*148*). Production of carnocin in modified MRS (MRS without meat extract or Tween 80; pH 6.5) was observed after 18 h growth at 25°C or after 148 h at 10°C, suggesting production during late logarithmic growth (*148*). Following growth, a cell-free supernatant was concentrated by membrane filtration, precipitated with 60% ammonium sulfate, resuspended, exhaustively dialyzed, and freeze-dried (*148*). The freeze-dried sample was analyzed by SDS-PAGE, and no distinct proteins bands were observed. However, inhibitory activity was observed in an area corresponding to 2,510 to 6,000 Da (*148*). Concentration on a membrane filter suggested that this protein was larger than 5,000 Da. No additional biochemical or genetic information about carnocin is available.

BACTERIOCINS OF CARNOBACTERIA

The newly named genus, *Carnobacterium* spp., includes nonaciduric, heterofermentative lactobacilli isolated from poultry, fish, and vacuum-packaged meat (*1,138*). While identification and classification of carnobacteria is relatively recent, bacteriocins associated with this genus have been reported (*1,3,17,18,124,138,139*) and will be discussed briefly in this section (Table 4).

Carnobacteriocins A1, A2, A3

Carnobacterium piscicola LV17 initially was isolated from meat (*1*). This organism produces two proteinaceous, heat stable (62°C, 30 min), bactericidal compounds (*1*). Bacteriocin activities of this organism appeared to be regulated by 61 and 75 kb nonconjugative plasmids (*1*). The spectrum of activity of these compounds is narrow and limited to other lactic acid bacteria (*1*). Recent studies have addressed the ability to mobilize the bacteriocin plasmids (*3*), as well as additional biochemical and genetic properties of these bacteriocins (*110,151*).

Recently, a procedure was devised which allows for the transfer of nonconjugative bacteriocinogenic plasmids between strains of carnobacteria (*3*). In this procedure, bacteriocin plasmids pCP40 and pCP49 of *C. piscicola* LV17 were subjected to two-stage conjugation. In the first stage, pAMβ1 was transferred conjugally to the bacteriocin-producing strain, *C. piscicola* LV17. In the second stage, the resulting transconjugants containing pCP40, pCP49, and pAMβ1 were conjugally mated with a bacteriocin-negative mutant strain of *C. piscicola*. The transconjugants from this mating expressed the bacteriocins in one of three ways: a) maximal bacteriocin activity (activity similar to wild-type producer) occurred when three plasmids were present; b) partial bacteriocin activity observed following transfer of only pCP40; c) maximal bacteriocin activity detected and the presence of a single, conintegrated plasmid (*3*). This procedure successfully demonstrates that desired bacteriocin genes can be transferred between strains without modification of the plasmids (*3*). Ultimately more efficient bacteriocin-producing strains can be developed for use in starter cultures using such techniques.

In another study, plasmid curing studies of the original producer strain, *C. piscicola* LV17, resulted in two strains, *C. piscicola* LV17A and LV17B (*151*). *C. piscicola* LV17A harbors a 75 kb plasmid (pCP49) that has been found to be responsible for production of and immunity to carnobacteriocins A1, A2, and A3, bacteriocins produced early in the growth cycle (*151*). Purification of the bacteriocins by HPLC and reverse phase HPLC revealed the presence of three active peaks. Edman degradation and subsequent analyses of the bacteriocins yielded similar N-terminal sequences for the first 25 amino acids of each bacteriocin. The molecular mass of carnobacteriocin A1 was estimated at 4,929 to 5,120 Da, as deduced by mass spectrometry (*151*). Mass spectrometry of carnobacteriocins A2 and A3 suggested molecular masses of 5,123 and 5,127 Da, respectively. A 23-mer oligonucleotide probe was constructed from the 25 N-terminal amino acids and used to probe pCP49. The probe hybridized with a 2.0 kb fragment which was subsequently cloned into pUC118, and the nucleotide sequence of the structural gene for carnobacteriocin A1 was determined (*151*).

Carnobacteriocins B1 and B2

C. piscicoloa LV17B, the other mutant strain of the original producer organism, produced two bacteriocins designated carnobacteriocins B1 and B2 (*110*). Following purification by HPLC, the molecular weights of the bacteriocins were determined by mass spectrometry and the amino acid sequence deduced. While carnobacteriocin B1 exhibited a molecular mass of 4,541 Da and consisted of 38 amino acids, carnobacteriocin B2 appeared as a 4,969 Da protein of 34 amino acids (*110*). Two oligonucleotide probes constructed from the amino acid sequences were used to probe pCP40, a 61 kb plasmid associated with production of these bacteriocins. Preliminary hybridization experiments with a pCP40 and a 24-mer probe for leucocin A (*63*) had indicated homology (*110*). Further analyses of the genetic determinants of carnobacteriocins B1, B2, and leucocin A indicated considerable homology among these bacteriocins (*110*). Additional biochemical and genetic information of these bacteriocins is forthcoming.

Carnocin U149

Carnocin U149 is produced by *Carnobacterium piscicola* isolated from fish (*138,139*). The bacteriocin is a heat stable (121°C, 15 min), bactericidal protein with activity towards carnobacteria, lactobacilli, pediococci, and lactococci (*138*). Following production during mid-logarithmic growth at 34°C, the bacteriocin was purified to homogeneity by ammonium sulfate precipitation, cation exchange chromatography, phenyl-superose chromatography, and reverse phase chromatography (*139*). SDS-PAGE of the purified protein suggested a molecular mass of 4,500 to 5,000 Da while mass spectrometry indicated a mass of 4,653 Da. Amino acid composition analysis of carnocin U149 revealed 35 to 37 residues, including lanthionine and constituted a molecular mass of 3,610 Da. Degradation of the bacteriocin resulted in the identification of seven N-terminal amino acids (*139*). No homology was detected between the known amino acids of carnocin U149 and other bacteriocins. Further research is under way to determine the primary sequence of this bacteriocin but may prove difficult because of the presence of lanthionine and/or other modified residues (*139*).

Unnamed bacteriocin

Carnobacterium piscicola LK5 was isolated from raw ground beef and found to produce a heat stable bacteriocin (*17*). Prelimi-

TABLE 4. *Characteristics of bacteriocins of carnobacteria.*

Bacteriocin	Producer	Molecular mass (daltons)	Stability	Sensitivity	Production parameters	Inhibitory spectrum	Gene locus	Mode of action	Reference
Carno-bacteriocin A1, A2, A3	*Carno-bacteria piscicola* LV17A	5,100 5,123 5,127 (mass spectro-metry)	62°C, 30 min	Proteolytic enzymes	Broth, early in growth	Lactic acid bacteria	75 kb plasmid	Not determined	*1,3,151*
Carno-bacteriocin B1, B2	*Carno-bacteria piscicola* LV17B	4,541 4,969 (mass spectrom-etry)	62°C, 30 min	Proteolytic enzymes	Broth, early in growth	Lactic acid bacteria	61 kb plasmid	Not determined	*1,3,110*
Carnocin U149	*Carno-bacteria piscicola*	3,610 (amino acid composition)	121°C, 15 min; pH<8	Proteolytic enzymes	Broth 24-48 h	Lactobacilli pediococci carno-bacteria	Not determined	Not determined	*138,139*
Unnamed	*Carno-bacteria piscicola* LK5	Not determined	100°C, 5 min	Trypsin, papin, pepsin, chymo-papain	Broth, 5°C	*L. mono-cytogenes*	Not determined	Not determined	*17,18*

nary characterization of the bacteriocin was based on resistance and/or sensitivity to various proteolytic enzymes (catalase, trypsin, papain, pepsin, chymopapain) and bactericidal activity towards several strains of *Listeria* spp. When co-cultured with *Listeria* spp., *C. piscicola* demonstrated considerable inhibition *in vitro*, presumably due to the effects of the bacteriocin (*17*). Additional experiments were performed in which the producer isolate effectively inhibited *L. monocytogenes* in ground beef, ultra-high temperature (UHT) milk, dog food, crabmeat, creamed corn, and frankfurters at refrigerated temperatures (*18*). These studies clearly identify the usefulness of bacteriocin-producing carnobacteria in food systems to inhibit foodborne pathogens.

BACTERIOCINS OF LACTOBACILLI

Antagonism by lactobacilli has been attributed to metabolic end products such as acid, lactoperoxidase, diacetyl, and hydrogen peroxide (*23,76*). Elimination of these inhibitory compounds from sample preparations has facilitated the detection of bactericidal proteins. Lactobacilli that produce bacteriocins have been cultured from naturally fermented dairy products, nondairy fermentations (plant and meat), starter cultures, and plant, animal, or human isolates. Studies of bacteriocinogenicity within the lactobacilli have focused on both biological and genetic properties (Table 5).

Bacteriocins of nondairy lactobacilli

Fermenticin. In 1961, fermenticin was isolated from *Lb. fermenti* cultures (*34*). Fermenticin is unaffected by pH, retained by dialysis membranes, precipitated by ammonium acetate, sensitive to trypsin and pepsin, resistant to heat, urea, or lysozyme, and contains a carbohydrate moiety (*35*). Purification of the bacteriocin from cell culture supernatant was accomplished by dialysis, chromatography on Sephadex G-100, and gel filtration. The bacteriocin contains 16 amino acids, 4 sugars, hexosamine, and phosphorus (*35*). No additional biochemical data are available. The genetic determinants for fermenticin production are unknown.

Plantaricin A and plantacin B. Strains of *Lb. plantarum*, a culture associated with silage and/or vegetable fermentations, produce the bacteriocins plantaricin A and plantacin B (*26*). Plantaricin A is produced by *Lb. plantarum* strain C-11 and is a bactericidal protein with activity restricted to other lactic acid bacteria. The molecular mass of plantaricin A is estimated at greater than 6,000 Da since the protein is retained in dialysis tubing. Activity of plantaricin A is unaffected by heating at 100°C for 30 min or pH adjustment 4 to 6.5. Plantaricin A is produced during the mid-logarithmic phase of growth with loss of activity during late log phase. Mutants of *Lb. plantarum* lacking plantaricin A activity show identical plasmid profiles to the parental strain. Plasmid curing treatments applied to *Lb. plantarum* C-11 did not alter the phenotype of the producer organism suggesting that plasmid DNA may not code for plantaricin A (*26*).

Plantacin B, a bacteriocin-like inhibitor produced by *Lb. plantarum* NCDO 1193, is a protein with a narrow spectrum of activity against other strains of *Lb. plantarum*, *Lb. mesenteroides*, and *P. damnosus* (*150*). Addition of lipase and α-amylase substantially reduced the inhibitory activity of plantacin B, suggesting that plantacin B was a protein complexed with carbohydrate and lipid moieties. The mode of lethal action, conditions favoring production in broth, and the basis for plantacin B production were not examined (*150*).

Sakacin A. Lb. sake is associated with fermented sausages and produces the bacteriocins, sakacin A, sakacin M, and lactocin S. Both sakacins A and M inhibit other lactobacilli, as well as *L. monocytogenes* (*125-128*). Lactocin S inhibits strains of lactic acid bacteria (*93,94*). Sakacin A is produced by *Lb. sake* 706, is heat stable (100°C, 20 min), produced during the mid- or late-logarithmic growth phase in liquid medium, and associated with a 27.7 kb plasmid (*126*). A bacteriocin-negative (bcn⁻) mutant was obtained by acriflavin treatment. Immunity and production of sakacin A accompanied loss of the plasmid, suggesting that both determinants were located on the plasmid. In a more recent study, sakacin A and *Lb. sake* 706 were evaluated for bactericidal activity towards *L. monocytogenes* on agar, in broth, and in two meat systems (pasteurized minced beef, and fresh mettwurst; *123,125*). Experiments with MRS agar and MRS broth demonstrated sakacin A activity towards various strains of *L. monocytogenes*. When assessed for activity in a food system, sakacin A was less effective against the pathogen (*125*). Meat systems may not support the bactericidal activity of sakacin A towards *L. monocytogenes* because of adsorption of the bacteriocin to meat or fat particles, the

TABLE 5. *Characteristics of bacteriocins of lactobacilli.*

Bacteriocin	Producer	Molecular mass (daltons)	Stability	Sensitivity	Production parameters	Inhibitory spectrum	Gene locus	Mode of action	Reference
Fermenticin	*Lactobacillus fermenti*	Not determined	pH; heat (96°C, 30 min); urea; lysozyme	Trypsin, pepsin	Supernates of overnight broth cultures	Lactobacilli	Not determined	Not determined	*34,35*
Plantaricin A	*Lactobacillus plantarum* C-11	>8,000 (dialysis)	Heat (100° C, 30 min); pH 4-6.5	Protease	Mid-log growth in broth culture	Lactobacilli, pediococci, leuconostocs lactococci	Not determined	Not determined	*26*
Plantacin B	*Lactobacillus plantarum* NCDO1103	Not determined	Not determined	Lipase, amylase, pronase, pepsin, trypsin, chymo-trypsin	Produced on agar, diffusable through agar	*Lb. plantarum*, *Leuconostoc mesen-teroides*, *P. damnosus*	Not determined	Not determined	*150*
Sakacin A	*Lactobacillus sake* 706	Not determined	Heat (100° C, 20 min); -20°C	Trypsin, pepsin	Broth; mid-late log growth at 25°C	Leuconostocs lactobacilli, enterococci, *L. mono-cytogenes*	27.7 kb plasmid	Not determined	*123,125, 126*
Sakacin M	*Lactobacillus sake* 148	4,640 (gel filtration)	80°C, 60 min; 150°C 9 min	Trypsin, pepsin, papain, proteases XIV, II	Semi-synthetic medium, 32°C	Lactobacilli, leuconostocs, carnobacteria *L. mono-cytogenes*, *S. aureus*	Not determined	Bacterio-static; not determined	*127,128*
Sakacin P	*Lactobacillus sake* LTH673	3,000-5,000 (SDS-PAGE and amino acid sequence)	Pepsin heat (100° C, 7 min	Proteinase K, trypsin	MRS broth (pH 6.5)	Lactobacilli, leuconostocs, carnobacteria enterococci, *Brochothrix thermosphacta*	Not determined	Not determined	*141*
Lactocin S	*Lactobacillus sake* L45	<13,700 (gel filtration)	heat (100° C, 60 min)	Protease, trypsin	Broth without Tween 80; late log growth	Pediococci, leuconostocs lactobacilli	50 kb plasmid	Possible attachment to cell membrane of sensitive cells	*93,94*
Curvacin A	*Lactobacillus curvatus* LTH1174	3,000-5,000 (SDS-PAGE) amino acid sequence	pepsin heat (100° C, 3 min)	Proteinase K, trypsin	MRS broth (pH 6.5)	Lactobacilli, leuconostocs, carnobacteria *Listeria* spp., micrococci, staphylococci	Not determined	Not determined	*141*
Brevicin	*Lactobacillus brevis* 37	>10,000 (ultrafiltra-tion)	pH 1-11; heat (121° C, 60 min)	Pronase E, trypsin; chloroform; pH>12 at 25 C	Supernatant of broth cultures	Pediococci, leuconostocs lactobacilli, *N. coralina*	Not determined	Not determined	*112*
Caseicin 80	*Lactobacillus casei* B80	40,000-42,000 (gel filtration)	pH<5.0	Pronase E, trypsin; heat (>60 C); pH>5.0	Inducible with mitomycin C in synthetic medium with peptone dialysate	*Lb. casei*	Not determined	Not determined	*111,112*
Plantaricin BN	*Lactobacillus plantarum* BN	>10,000 (SDS-PAGE)	heat (100° C, 5 min; 60°C, 10 min)	Not determined	BHI agar, pH 6.9, 15 C	*Lb. sake*	Not determined	Bactericidal; not determined	*83*

TABLE 5. *Cont.*

Bacteriocin	Producer	Molecular mass (daltons)	Stability	Sensitivity	Production parameters	Inhibitory spectrum	Gene locus	Mode of action	Reference
Bavaracin MN	*Lactobacillus bavaricus* MN	22,600 (SDS-PAGE)	heat (100° C, 5 min; 60°C, 10 min)	Not determined	APT agar, pH 6.5, 30 C	*Lb. sake*	Not determined	Bactericidal; not determined	*83*
Lactocin 27	*Lactobacillus helveticus* LP27	12,400 (SDS-PAGE)	Ficin; chloroform; SDS; heat (100°C, 60 min)	Trypsin, pronase	Broth cultures, diffused in agar	*Lb. acidophilus*, *Lb. helveticus*	Chromosomal; no detectable plasmids	Efflux of potassium ions, influx of sodium ions	*143,144*
Helveticin J	*Lactobacillus helveticus* 481	37,000 (SDS-PAGE and genetic data)	Lipase; 0.1% SDS; lysozyme	Heat, ficin, pronase, trypsin, pepsin, proteinase K, subtilisin; 6M guanidine HCl; 0.2% β-mercaptoethanol	Late log-early stationary growth; controlled pH 5.5 in broth under anaerobic conditions	*Lb. bulgaricus*, *Lb. lactis*, *Lb. helveticus*	Chromosomal	Not determined	*72,73*
Helveticin V-1829	*Lactobacillus helveticus* V-1829	Not determined	Dissociating agents; mild heat (45°C, 120 min); pH 2.5-6.5	Heat (50°C, 30 min); proteinase K, ficin, trypsin, pronase, pH>7	MRS broth (pH 5.5), milk under anaerobic conditions; during log growth	Lactobacilli	Chromosomal	Not determined	*149*
Lactacin F	*Lactobacillus acidophilus* 11088	2,500 (SDS-PAGE and genetic data)	SDS; heat (121°C, 15 min)	Proteinase K, trypsin, ficin, subtilisin	Controlled pH 7.0 in broth	Lactobacilli, *E. faecalis*	Episomal	Not determined	*96-98*
Lactacin B	*Lactobacillus acidophilus* N2	8,100 (SDS-PAGE)	1% SDS; 8 M urea; heat (121° C, 3 min), -20°C, β-mercapto-ethanol	Proteinase K, pronase	Controlled pH 6.0 in semi-defined media; early stationary growth	Lactobacilli	Chromosomal; no detectable plasmids	Not determined	*6,7,99*

presence of endogenous enzymes, or loss of activity over time (*125*).

Sakacin M. Lb. sake 148 was isolated from Spanish dry fermented sausage (*127*). The inhibitory compound, designated sakacin M, is maximally produced in a semi-synthetic medium supplemented with 1.5% tryptone during growth at 32°C (*128*). Following growth, the cell-free supernatant was concentrated by lyophilization, resuspended in 1 M urea, pH 5.6, and partially purified by gel filtration. Elution of an active fraction corresponded to a molecular weight of 4,640 Da. While SDS-PAGE revealed a single diffuse protein band, no size estimations of the compound were made (*128*). Inhibitory activity of the partially purified compound was diminished by treatments with 1 mg/ml of trypsin, pepsin, papain, and proteases XIV and II after 12 h, 37°C. Both the crude and partially purified compound were heat stable as evidenced by treatments at 80°C for 60 min and 150°C for 9 min (*128*). Partially purified sakacin M exhibits a bacteriostatic mode of action against sensitive cells since treatments with crude extracts resulted in some growth of lactobacilli, leuconostocs, carnobacteria, *L. monocytogenes*, and *S. aureus* after 16 h (*128*). Presently, no information about the genetic determinants for sakacin M is available.

Sakacin P. Lb. sake LTH673, a meat isolate, produces a bactericidal protein that inhibits lactobacilli, including meat starter cultures and spoilage organisms, leuconostocs, carnobacteria, enterococci, *Brochothrix thermosphacta*, and *Listeria* spp. (*141*). Designated sakacin P, this bacteriocin is sensitive to proteinase K and trypsin but insensitive to pepsin and heat (100°C, 7 min). Sakacin P was purified from cell-free supernatants of MRS broths (pH 6.5) by ammonium sulfate precipitation, cation-exchange, hydrophobic interaction, and reversed-phase chromatography. Amino acid composition and sequence analysis revealed a compound consisting of 36 to 41 residues, a finding consistent with SDS-PAGE data indicating a mass of 3,000 to 5,000 Da (*141*). No additional genetic data are available at this time.

Lactocin S. Lactocin S, the bacteriocin produced by *Lb. sake* L45 also has been identified and characterized (*93,94*). Lactocin S is a heat stable protein active against *Pediococcus*, *Leuconostoc*, and *Lactobacillus* (*93*). The bacteriocin is produced during late-exponential growth in liquid media. Lactocin S activity was increased 25- to 30-fold by concentration with ammonium sulfate. Purification of the protein was accomplished by ion-exchange

chromatography, phenyl-sepharose chromatography, gel filtration, and reverse-phase chromatography. Molecular weight determination of crude lactocin S suggested a molecular mass of 30,000 (*94*). Gel filtration of the partially purified protein identified an active fraction less than 13,700 Da (*93*). The discrepancies in molecular weight determination were attributed to the ability of the protein to associate with other substances or with itself before purification (*94*). Amino acid analysis indicated that lactocin S contains 33 amino acids. Sequencing from the C-terminal end identified 28 amino acid residues; 50% are nonpolar amino acids, suggesting a hydrophobic protein (*94*). The authors speculated that the hydrophobic nature of lactocin S may be responsible for attachment of the bacteriocin to the cell membrane of sensitive cells (*94*). Additional experiments revealed that immunity and production of lactocin S were regulated by an unstable 50 kb plasmid (*94*). As plasmid instability is not desirable, chromosomal integration may be a useful alternative for stabilizing bacteriocin production in this organism.

Curvacin A. Another meat isolate, *Lb. curvatus* LTH1174, produces the bacteriocin, curvacin A (*141*). The spectrum of activity of this bacteriocin is directed towards other lactobacilli, leuconostocs, carnobacteria, *L. monocytogenes*, *L. ivanovii*, as well as weakly inhibiting micrococci and staphylococci (*141*). Like sakacin P, curvacin A is destroyed by proteinase K and trypsin but stable when treated with pepsin or heat (100°C, 3 min). Following production in MRS broth (pH 6.5), curvacin A was purified to homogeneity by ammonium sulfate precipitation, cation-exchange, hydrophobic interaction, and reversed-phase chromatography. SDS-PAGE analysis of the purified protein indicated a molecular mass of 3,000 to 5,000 Da. Amino acid sequence information identified 30 residues while composition analysis suggested 38 to 41 (*141*). Both sakacin P and curvacin A are hydrophobic proteins that share a Tyr-Gly-Asn-Gly-Val sequence (*141*). The similarities in sequence analysis and inhibitory spectrum may suggest a conserved region (*141*). Elucidation of this information awaits additional genetic data.

Brevicin 37 and caseicin 80. In another study, *Lb. brevis* B37 and *Lb. casei* B80 were isolated from plants or fermenting material (*112*). *Lb. brevis* produces the bacteriocin, brevicin 37, which is active against other lactic acid bacteria (*Pediococcus* spp., *Leuconostoc* spp., *Lactobacillus* spp.) and *Nocardia coralina*. The protein is stable at various pH conditions (pH 1 to 11), is heat stable (121°C for 1 h), can be concentrated by lyophilization, and is retained on a 10,000 molecular weight cutoff (MWCO) membrane (*112*).

The same study showed that caseicin 80, produced by *Lb. casei* B80, is a heat sensitive protein with a narrow spectrum of activity (*111*). Caseicin 80 exerts a weak bactericidal effect on sensitive cells (*111*). Growth of the producer culture in a synthetic medium, containing peptone dialysate, and treatment with mitomycin C yielded a 5- to 7-fold increase in bacteriocin production (*111*). The bacteriocin was concentrated by ultrafiltration using a Sartorius system. Purification was accomplished by filtration through a 10,000 MWCO membrane, dialysis, and cation-exchange chromatography on Cellulose SE-23 and gel filtration on Superose (*111*). Gel filtration experiments yielded a protein with a molecular size of 40,000 to 42,000 Da. Caseicin activity was detected at pH 4.5 during isoelectric focusing, suggesting it had an isoelectric point of 4.5. Protein stability was confirmed at pH <5.0 (*111*). Caseicin 80 is the first reported inducible bacteriocin of the lactic acid bacteria.

Plantaracin BN and bavaricin MN. Lb. plantarum BN and *Lb. bavaricus* MN were isolated from retail beef (*83*). Initially, production parameters for these bacteriocins were performed on solid media On brain heart infusion (BHI) agar, maximal production of plantaricin BN occurred at pH 6.9, 15°C. Bavaricin MN was produced maximally on APT agar, pH 6.5, 30°C (*83*). Plantaricin production in broth was variable and inconsistent while bavaracin was produced in all broths examined. Crude preparations of plantaricin BN and bavaricin MN were obtained from freeze-thaw extracts of agar, assayed for heat stability, and molecular mass estimated (*83*). Both plantaracin BN and bavaracin MN were heat stable (100°C, 5 min or 60°C, 10 min) proteins with molecular masses of greater than 10,000 and 22,600 Da, respectively (*83*). Genetic information about these compounds is not yet available.

Bacteriocins of dairy lactobacilli

Lactocin 27. Lactocin 27 was isolated from the supernatant of broth cultures of *Lb. helveticus* LP27 and is a heat stable, proteinaceous compound that diffuses in agar, is nondialyzable, and exhibits bacteriostatic activity towards sensitive lactobacilli (*143,144*). Lactocin 27 was purified by treatments with chloroform, chromatography on Sephadex G-200, and chromatography with Sephadex G-25 (*144*). Dissociation in SDS released the active moiety, a 12,400 Da molecular weight glyco-protein (*143*). The action of lactocin 27 on sensitive cells results in an efflux of potassium ions and influx of sodium ions without interfering with DNA or RNA synthesis. The loss of potassium ions suggests that this bacteriocin acts on the cell membrane (*144*). Production of this bacteriocin appears to be regulated by chromosomal determinants since no plasmids were detected (*144*).

Helveticin J. More recently, the bacteriocin helveticin J was identified in *Lb. helveticus* 481 (*72*). Helveticin J is sensitive to several proteolytic enzymes and heat, exhibits a bactericidal mode of action limited to related lactobacilli, is purified from culture supernatants, and is encoded by chromosomal determinants (*72*). Purification of helveticin J was as follows: *Lb. helveticus* was grown in an anaerobic fermenter (22 h at 37°C, controlled pH 5.5); crude helveticin J was harvested from the fermenter, adjusted to pH 3.0 and then precipitated with 50% ammonium sulfate. The molecular mass of crude helveticin J appeared to be greater than 300,000 Da, due to its retention on XM 300 ultrafiltration membranes. The ammonium sulfate pellet was resuspended, dialyzed, reprecipitated, solubilized in 6% SDS, and concentrated. Helveticin J was subjected to gel chromatography (Sephadex G-200-50) and SDS-PAGE. Both methods identified a single protein band corresponding to 37,000 Da (*72*). In its crude form, helveticin J appears as an aggregated protein of 300,000 Da. In the presence of SDS, the bacteriocin dissociates to a monomeric form of 37,000 Da, thereby explaining the size discrepancies encountered during purification (*72*).

Nucleotide sequences of both the structural and immunity genes for helveticin J have been identified (*73*). λgt11 and λEMBL3 libraries were made from whole genomic DNA of *Lb. helveticus* 481. *E. coli* Y1089 was infected with the λgt11 phage library and induced, producing two β-galactosidase-helveticin J fusion proteins. The phage that produced the fusion proteins, HJ1 and HJ4, harbored 300 bp and 600 bp inserts of genomic DNA respectively but did not demonstrate production of active bacteriocin. Insert DNA from the two phage were used as probes to screen intact genomic DNA and the λEMBL3 library. The DNA from HJ1 and HJ4 hybridized to each other, to intact genomic DNA from *Lb. heleveticus* 481, and to a recombinant phage (Hlv3) associated with the λEMBL3 library. Subcloning of the DNA from Hlv3 into phage M13 revealed a contiguous 3364 bp region. Sequence information from the region determined the presence of four ORFs. The region consisted of a 3' end of ORF1, a noncoding region and a putative promoter between ORF1 and ORF2, an ORF3, located 30 bp downstream from the termination codon of ORF2, and the 5' end of ORF4. ORF2 is thought to encode for an 11,808 Da protein with a pI of 6.81, possibly a signal peptide. The DNA inserts of phages HJ1 and HJ4 hybridized within ORF3. A 37,511 Da protein with a pI of 6.03 is encoded by ORF3 in agreement with the molecular mass of 37,000 for helveticin J (*72*); however, the significance of ORF4 is not known (*73*).

Cloning experiments were performed with a 4 kb fragment from *Lb. helveticus* 481-C DNA (*73*). The 4 kb fragment was inserted into pGK12, forming pTRK135. Transformation of *E. coli* with pTRK135 was successful, but helveticin J activity was not detected in broth supernatant or cell extracts (*73*). pTRK135 was electroporated into strains of *Lb. acidophilus* and *Lb. fermentum* with variable results. Some isolates deleted all or parts of the plasmid while a single strain, *Lb. acidophilus* NCK64, harbored the intact plasmid. *Lb. acidophilus* NCK64 expressed a protein with identical spectrum of activity and heat sensitivity to helveticin J (*73*). This report demonstrated the first successful cloning and expression of a bacteriocin from lactobacilli.

Helveticin V-1829. A dairy isolate, *Lb. helveticus* 1829, produces the newly recognized bacteriocin, helveticin V-1829 (*149*). Following the elimination of acids, hydrogen peroxide, and bacteriophage from crude preparations, helveticin V-1829 was found to be a heat labile (50°C, 30 min), bactericidal protein with inhibitory activity against other lactobacilli (*149*). Production of the crude bacteriocin occurs in MRS broth or milk with maximal production during logarithmic growth in MRS broth (pH 5.5) under anaerobic conditions (*149*). Following treatments with various dissociating agents, activity of the crude bacteriocin did not increase, suggesting that the bacteriocin is not produced in an aggregated form or these agents are unable to dissociate (*149*). Helveticin V-1829 was partially purified following growth in a semidefined MRS medium. A cell-free filtrate was treated sequentially with ammonium sulfate to 30 and 60% saturation at 4°C. The resulting precipitate was resuspended and dialyzed (*149*). The partially purified preparation was inactivated by treatments with proteinase K, ficin, trypsin, pronase, heat, and above pH 7.0. Between pH 2.5 and 6.5 and mild heat treatments (45°C, 120 min), helveticin V-1829 activity was retained (*149*).

Lb. helveticus 1829 harbors no detectable plasmids, suggesting that determinants for bacteriocin production are located chromosomally (*149*). To determine if any genetic similarities existed between the two known helveticins, DNA homology studies were performed. Specific primers to the structural gene of helveticin J (*hlv*) were utilized in polymerase chain reaction (PCR) to amplify a 1 kb probe from whole genomic DNA of *Lb. helveticus* 481. The *hlv* specific probe did not hybridize to genomic DNA of Lb. helveticus 1829, indicating that no significant homology exists between helveticins J and V-1829 (*149*).

Lactacin F. Another dairy lactobacillus, *Lb. acidophilus* 11088, produces the bacteriocin lactacin F (*96*). Lactacin F is sensitive to proteolytic enzymes (proteinase K, trypsin, ficin, subtilisin), is stable in SDS, and is heat stable (121°C for 15 min; *96*). It inhibits *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. delbrueckii* subsp. *lactis* (formerly *Lb. leichmannii*), *Lb. helveticus*, *Lb. acidophilus*, *Lb. fermentum*, and one strain of *E. faecalis* (*96*). Maximum production of lactacin F occurs during early stationary phase of growth when the broth culture is maintained at pH of 7.0 (*96*). Lactacin F can be concentrated from a cell-free supernatant by addition of ammonium sulfate (40% saturation). Lactacin F was detected in the precipitate, supernatant, and surface flocculates (*96*). Most of the lactacin F activity was found in surface flocculate and this crude sample was used in subsequent experiments.

Crude lactacin F was applied to ultrafiltration experiments, electron microscopy studies, and purification procedures. Ultrafiltration experimentations utilized 300 and 100 kDa membranes. Rentention of the protein on the 100 kDa membrane increased lactacin F activity to 14-fold (*97*). Following ultrafiltration, crude lactacin F was subjected to gel filtration chromatography and molecular mass was estimated to be approximately 180 kDa (*97*). Transmission electron microscopy revealed micelle-like particles of 25-50 nm in diameter (*97*). HPLC purification followed by SDS-PAGE revealed lactacin F activity was retained in a 2500 Da band (97). Migration of the bacteriocin to 2500 Da in SDS-PAGE gels did not correlate with amino acid composition analysis indicating a total of 56 amino acids (*97*). Sequence information of the purified protein demonstrated 25 N-terminal amino acids: NH_2-Arg-Asn-Asn-Trp-Gln-Thr-Asn-Val-Gly-Gly-Ala-Val-Gly-Ser/Cys-Ala-Met-Ile-Gly-Ala-Thr-Val-Gly-Gly-Thr-Ile. . . (*97*). Preliminary peptide structure analysis indicated that lactacin F is composed of a hydrophilic N-terminal portion and a hydrophobic core (*97*). The hydrophobicity of lactacin F may explain flocculation during treatments with ammonium sulfate, the tendency to bind during HPLC chromatography, and the abnormal migration of the protein in SDS-PAGE (*96,97*). Subsequent genetic analysis of the lactacin F gene identified 57 amino acids (*96*), including the 25 N-terminal amino acids, and corresponded to previous composition data in which the active protein contains 56 amino acids (*96*). Genetic information also indicated the presence of an extension sequence, suggesting that lactacin F may be processed as a prepeptide and undergoes proteolytic cleavage to an active protein. The amino acid sequence of the N-terminal extension of lactacin F consists of 18 amino acids: a positively charged lysine residue at the N terminus, a hydrophobic core region (leu-ala-val-val-val), and a C terminus (val-gly-gly; *97*). The significance of this extension sequence was not determined; however, it may be part of a signal sequence necessary for secretion of the active protein (*97*).

Genetic determinants for lactacin F production and immunity are encoded on an episomal element (*96-98*). The episome was capable of transferring the genes for production and immunity between plasmid DNA and the bacterial chromosome in *Lb. acidophilus*. The following strains were utilized in studies to clone and sequence the lactacin F structural gene: *Lb. acidophilus* 11088, the lactacin F producer laf$^+$ laf^r); *Lb. acidophilus* 88-C, a derivative of the producer strain that did not produce lactacin F (laf$^-$) but was resistant to the bacteriocin (laf^r); *Lb. acidophilus* 89, a transconjugant that did not produce bacteriocin or resistance (laf$^-$ laf^s); *Lb. acidophilus* T89, a stable transconjugant with ability to produce lactacin F (lac F$^+$) and immunity to it (laf^r); *Lb. acidophilus* T143, an unstable transconjugant capable of lactacin F production and resistance (laf$^+$laf^r) (*97,98*). The phenotypes of the *Lb. acidophilus* strains used in this study exhibit the phenotypes associated with intragenic conjugation and provide evidence for involvement of episomal elements and/or transient plasmids (*98*).

A 63-mer oligonucleotide probe, deduced from the N-terminus of the lactacin F amino acid sequence, was constructed and hybridized to a 2.2 kb fragment of pPM68, a 110 kb lactacin F related plasmid found in *Lb. acidophilus* T143 (*98*). The homologous 2.2 kb fragment was subcloned into pTRK159, a 10.3 kb *E. coli-Lactobacillus* shuttle-cloning vector. The resulting plasmid, pTRK162, was electroporated into the non-producing lactacin F variants, *Lb. acidophilus* 89 and 88C. Two transformants, *Lb. acidophilus* NCK368 and *Lb. acidophilus* NCK369, harbored the 2.2 kb fragment, produced lactacin F, and were immune to the bacteriocin. A series of subcloning experiments and use of a 20-mer oligonucleotide probe (also deduced from the amino acid sequence) localized the lactacin F structural gene to a 0.9 kb fragment (*98*). DNA sequence analysis was performed on this region. Included within the 0.9 kb fragment were the lactacin F structural gene and two ORFs. The lactacin F structural gene contained all 25 N-terminal amino acids of lactacin F identified previously through protein sequencing (*97*). A ribosomal binding site 54 bp upstream from the putative lactacin F structural gene suggested translation of a leader sequence or pre-lactacin F peptide (*98*). As with nisin, the pre-lactacin F peptide may be post-translationally processed to the active bacteriocin. In close proximity to and downstream from the lactacin F gene are 2 ORFs. One ORF, termed ORFX, may encode a protein of 32 amino acids. The significance of this protein was not discussed but may be associated with immunity or resistance determinants (*98*).

Lactacin B. Lactacin B is another bacteriocin produced by *Lb. acidophilus*, strain N2. Lactacin B is produced in semi-defined

medium (7) and on solid media (6), is sensitive to enzymatic treatments with proteinase K, is stable in chaotropic agents (SDS, urea), and heat stable (100°C for 3 min at pH 5; 6). It inhibits *Lb. delbrueckii* subsp. *lactis* (formerly *Lb. leichmannii*), *Lb. delbrueckii* subsp. *bulgaricus*, and *Lb. helveticus* (6). Lactacin B was partially purified by ion-exchange chromatography, ultrafiltration, and successive gel filtrations in SDS and urea (7). Gel filtration experiments indicated a molecular mass of approximately 6,500 Da for lactacin B. Analysis by SDS-PAGE yielded no detectable protein band; however, lactacin B activity was retained, suggesting that the protein was not present in sufficient amounts or could not be detected by silver stain (7).

In another study, a simpler purification protocol for lactacin B was devised (99). Following production of the bacteriocin in semi-defined media, culture supernates were lyophilized to concentrate the bacteriocin. The lyophilized supernate was then resuspended in 6 M urea, ultrafiltered through 50,000 and 3,000 MWCO membranes, and subjected to preparative isoelectric focusing (IEF). SDS-PAGE analysis of the protein identified a single band at a molecular mass corresponding to 8,100 Da (99). Differences in size estimates for lactacin B were attributed to several factors. Initially, migration and size of lactacin B in SDS-PAGE was based on migration of an active bacteriocin in unfixed gels and not on the presence of a visible band (7). The use of bacitracin as a standard and the lower fractionation limit of SDS-gel chromatography also may have resulted in an erroneous calculation of the molecular mass of lactacin B (7). Amino acid composition analysis of purified lactacin B indicated a molecular mass of 8,000 to 9,000 Da, a figure consistent with data obtained in SDS-PAGE (99). Additionally, the proportion of alanine and valine suggested that lactacin B is a highly hydrophobic protein and may account for the size discrepancies encountered in these studies (99).

Production of lactacin B appears to be encoded by genomic determinants since no plasmids were detected in *Lb. acidophilus* N2 (6). Treatment of the producer organism with nitrosoguanadine resulted in disruption of lactacin B activity, indicating that a single agent is responsible for inhibition (7,99). Until additional reports characterizing the genetic determinants of lactacin B are identified, precise size determinations cannot be made.

APPLICATIONS OF BACTERIOCINS AND/OR BACTERIOCIN-PRODUCING ORGANISMS IN FOOD SYSTEMS

The direct addition of bacteriocins or the use of bacteriocin-producing lactic acid bacteria associated with foods may provide a novel means of preserving foods and beverages from the detrimental effects of spoilage and/or pathogenic bacteria. As discussed throughout this review, researchers have identified bacteriocins of lactic acid bacteria that inhibit a broad range of gram-positive organisms, including the pathogens, *S. aureus*, *L. monocytogenes*, and *Cl. botulinum*. It is also possible that the spectrum of activity of these bacteriocins can be extended to gram-negative organisms by combining these compounds with chelating agents (134-136). For the most part, these studies have demonstrated the effectiveness of bacteriocins against undesirable bacteria *in vitro*. Several recent studies have indicated that, when produced by lactic acid bacteria as part of their fermentation or used directly in food systems (*in situ*), bacteriocins retain their antimicrobial activities (8,17,18,21,23,24,32,33,37,42,59,60,89, 90,101,102,125).

Of all the bacteriocins discussed in this review, only nisin has been granted GRAS (generally regarded as safe) status and is approved for use in the United States (43). The nisin-producing organism, *L. lactis* subsp. *lactis*, currently is used to manufacture ripened cheeses while nisin is used as an additive in processed cheese spreads. Given the spectrum of its inhibitory activity and stability, nisin should be applicable to other food systems. The approval and use of nisin has set a precedent for the consideration of other bacteriocins in food systems (24).

There are several factors to consider prior to directly adding bacteriocins to foods (24). For bacteriocins to be effectual in foods, the food's structure and composition must be evaluated. For example, fat content can affect activity of the bacteriocin. Studies have shown that bacteriocins, including nisin, may bind preferentially to fat, thereby interfering with activity against bacteria (24,125). Temperature, pH, and presence of exogenous enzymes (i e., proteases) are critical parameters for bacteriocin stability in foods or beverages. Because of the narrow pH ranges in which some bacteriocins are active, they should be used with foods or beverages with similar pH values that ensure maximal activity. Most bacteriocins of lactic acid bacteria are generally small, heat stable proteins, thereby favoring use in thermally processed or refrigerated foods. Since bacteriocins are proteinaceous compounds, they may be susceptible to endogenous proteases or proteinases present in foods (20) and should be evaluated carefully for retention of activity. Whereas degradation of these compounds is undesirable in food systems, inactivation by gastrointestinal enzymes, such as chymotrypsin or trypsin, is advantageous. Inactivation by these enzymes renders bacteriocins inert and may explain why no adverse effects have been associated with ingestion of bacteriocins (24). Extensive toxological studies, such as those carried out on nisin, will be needed before other bacteriocins are approved for use as food additives. In addition to broad activity, stability, and nontoxicity, the use of bacteriocins should be practical, inexpensive, and should not affect organoleptic characteristics of foods or beverages to which they are added (24).

Bacteriocins may also be present in established food systems (i.e., ripened cheeses, yogurt, sausages, pickles) as by-products of starter culture fermentations. Starter cultures and their bacteriocins have been discussed earlier in this review (8,11,42,93,101,106,126). In some studies, producer organisms were reintroduced into food systems and monitored for bacteriocin production (8,18,33,42,59,60,89,90, 108,126). Results of these studies indicate that bacteriocinogenic starter cultures may be useful in preventing or controlling contamination by undesirable bacteria during fermentation. Hence, the addition of bacteriocin-producing food isolates to various food systems is an acceptable alternative to the direct addition of bacteriocins (24,67).

Another application in which bacteriocins may be utilized involves the cloning of production and immunity determinants. Earlier in this review, several studies reported the cloning of bacteriocin genes into nonbacteriocinogenic lactic acid bacteria (73,98) and *E. coli* (66,147). Genes for bacteriocin production and immunity were once thought to be a useful alternative to clinical antibiotic resistance markers for the development of food grade cloning vectors (67). Today, the possibilities exist that selected starter cultures can be improved with these same techniques. Not only will these organisms maintain the desired flavor and texture components for which they were selected, but they also may be

engineered to produce several bacteriocins active against a variety of pathogens or spoilage organisms. Additionally, knowledge of the amino acid structure of these compounds may enable scientists to develop hybrid bacteriocin molecules (*88*). In such cases, desirable aspects of several bacteriocins could be incorporated, thereby generating compounds with broad spectrum inhibition, specific lethality, or target-cell specificity. Considerable research and testing will be required before genetically engineered starter cultures or specially designed bacteriocin molecules are considered safe or stable enough to be utilized in commercially fermented foods. The direct addition of bacteriocins, the use of bacteriocinogenic starter cultures, or the development of genetically manipulated starter cultures with bacteriocin genes are potential alternatives for improving the overall quality and safety of foods.

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REFERENCES

1. Ahn, C., and M. E. Stiles. 1990. Plasmid-associated bacteriocin production by a strain of *Carnobacterium piscicola* from meat. Appl. Environ. Microbiol. 56:2503-2510.
2. Ahn, C., and M. E. Stiles. 1990. Antibacterial activity of lactic acid bacteria isolated from vacuum-packaged meats. J. Appl. Bacteriol. 69:302-310.
3. Ahn, C., and M. E. Stiles. 1992. Mobilization and expression of bacteriocin plasmids from *Carnobacterium piscicola* isolated from meat. J. Appl. Bacteriol. 73:217-228.
4. Allison, G., K. Roy, and M. Stiles. 1992. Cloning of the leucocin A gene into *Escherichia coli*, abstr. P-12, p. 326. Abstr. 92nd Annu. Meet. Am. Soc. Microbiol., 1992.
5. Barefoot, S. F., K. M. Harmon, D. A. Grindstead, and C. G. Nettles. 1992. Bacteriocins, molecular biology. pp. 191-202. *In* J. L. Lederberg (ed.), Encyclopedia of microbiology, vol. 1. Academic Press, Inc., New York.
6. Barefoot, S. F., and T. R. Klaenhammer. 1983. Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 45:1808-1815.
7. Barefoot, S. F., and T. R. Klaenhammer. 1984. Purification and characterization of the *Lactobacillus acidophilus* bacteriocin lactacin B. Antimicrob. Agents Chemother. 26:328-334.
8. Berry, E. D., M. B. Liewen, R. W. Mandigo, and R. W. Hutkins. 1990. Inhibition of *Listeria monocytogenes* by bacteriocin-producing *Pedicoccus* during the manufacture of fermented semidry sausage. J. Food Prot. 53:194-197.
9. Bhunia, A. K., and M. C. Johnson. 1992. A modified method to directly detect in SDS-PAGE the bacteriocin of *Pediococcus acidilactici*. Lett. Appl. Microbiol. 15:5-7.
10. Bhunia, A. K., M. C. Johnson, and B. Ray. 1987. Direct detection of an antimicrobial peptide of *Pediococcus acidilactici* in sodium doedecyl sulfate-polyacrylamide gel electrophoresis. J. Indust. Microbiol. 2:319-322.
11. Bhunia, A. K., M. C. Johnson, and B. Ray. 1988. Purification, characterization and antimicrobial spectrum of a bacteriocin produced by *Pediococcus acidilactici*. J. Appl. Bacteriol. 65:261-268.
12. Bhunia, A. K., M. C. Johnson, B. Ray, and E. L. Belden. 1990. Antigenic property of pediocin AcH produced by *Pediococcus acidilactici* H. J. Appl. Bacteriol. 69:211-215.
13. Bhunia, A. K., M. C. Johnson, B. Ray, and N. Kalchayanand. 1991. Mode of action of pediocin AcH from *Pediococcus acidilactici* H on sensitive bacterial strains. J. Appl. Bacteriol. 70:25-33.
14. Biswas, S. R., P. Ray, M. C. Johnson, and B. Ray. 1991. Influence of growth conditions on the production of a bacteriocin, pediocin AcH, by *Pediococcus acidilactici* H. Appl. Environ. Microbiol. 57:1265-1267.
15. Bradley, D. E. 1967. Ultrastructure of bacteriophage and bacteriocins. Bacteriol. Rev. 31:230-314.
16. Bruno, M. E. C., A. Kaiser, and T. J. Montville. 1992. Depletion of proton motive force by nisin in *Listeria monocytogenes* cells. Appl. Environ. Microbiol. 58:2255-2259.
17. Buchanan, R. L., and L. A. Klawitter. 1992. Characterization of a lactic acid bacterium, *Carnobacterium piscicola* LK5, with activity against *Listeria monocytogenes* at refrigeration temperatures. J. Food Safety 12:199-217.
18. Buchanan, R. L., and L. A. Klawitter. 1992. Effectiveness of *Carnobacterium piscicola* LK5 for controlling the growth of *Listeria monocytogenes* in refrigerated foods. J. Food Safety 12:219-236.
19. Buchman, G. W., S. Banerjee, and J. N. Hansen. 1988. Structure, expression and evolution of a gene encoding the precursor of nisin, a small protein antibiotic. J. Biol. Chem. 263:16260-16266.
20. Chassy, B. M. 1987. Prospects for the genetic manipulation of lactobacilli. FEMS Microbiol. Rev. 46:297-312.
21. Chung, K.-T., J. S. Dickson, and J. C. Crouse. 1989. Effects of nisin on growth of bacteria attached to meat. Appl. Environ. Microbiol. 55:1329-1333.
22. Daba, H., S. Pandian, J. F. Gosselin R. E. Simard, J. Huang, and C. Lacroix. 1991. Detection and activity of a bacteriocin produced by *Leuconostoc mesenteroides*. Appl. Environ. Microbiol. 57:3450-3455.
23. Daeschel, M. A. 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technol. 43:164-167.
24. Daeschel, M. A. 1990. Applications of bacteriocins in food systems, pp. 91-115. *In* D. D. Bills and S. Kung (ed.), Biotechnology and food safety. Butterworth-Heinemann, Boston.
25. Daeschel, M. A., and T. R. Klaenhammer. 1985. Association of a 13.6-megadalton plasmid in *Pediococcus pentosaceus* with bacteriocin activity. Appl. Environ. Microbiol. 50:1538-1541.
26. Daeschel, M. A., M. C. McKenney, and L. C. McDonald. 1990. Bacteriocidal activity of *Lactobacillus plantarum* C-11. Food Microbiol. 7:91-98.
27. Davey, G. P. 1981. Mode of action of diplococcin, a bacteriocin from *Streptococcus cremoris* 346. N. Zeal. J. Dairy Sci. Technol. 16:187-190.
28. Davey, G. P. 1984. Plasmid associated with diplococcin production in *Streptococcus cremoris*. Appl. Environ. Microbiol. 48:895-896.
29. Davey, G. P., and L. E. Pearce. 1980. The use of *Streptococcus cremoris* strains cured of diplococcin production as cheese starters. N. Zeal. J. Dairy Sci. Technol. 15:51-57
30. Davey, G. P., and L. E. Pearce. 1982. Production of diplococcin by *Streptococcus cremoris* and its transfer to nonproducing group N streptococci. pp. 221-224. *In* D. Schlessinger (ed.), Microbiology-1982. American Society for Microbiology, Washington, DC.
31. Davey, G. P., and B. C. Richardson. 1981. Purification and some properties of diplococcin from *Streptococcus cremoris* 346. Appl. Environ. Microbiol. 41:84-89.
32. Degnan, A. J., and J. B. Luchansky. 1992. Influence of beef tallow and muscle on the antilisterial activity of pediocin AcH and lipsome-encapsulated pediocin AcH. J. Food Prot. 55:552-554.
33. Degnan, A. J., A. E. Yousef, and J. B. Luchansky. 1992. Use of *Pediococcus acidilactici* to control *Listeria monocytogenes* in temperature-abused vacuum-packaged wieners. J. Food Prot. 55:98-103.
34. DeKlerk, H. C., and J. N. Coetzee. 1961. Antibiosis among lactobacilli. Nature (London) 192:340-341.
35. DeKlerk, H. C., and J. A. Smit. 1967. Properties of a *Lactobacillus fermenti* bacteriocin. J. Gen. Microbiol. 48:309-316.
36. Delves-Broughton, J. 1990. Nisin and its uses as a food preservative. Food Technol. 44:100-112.
37. Delves-Broughton, J., G. C. Williams, and S. Wilkinson. 1992. The use of the bacteriocin, nisin, as a preservative in pasteurized liquid whole egg. Lett. Appl. Microbiol. 15:133-136.
38. Dobrzanski, W. T., J. Bardowski, W. Kozak, and J. Zajdel. 1982. Lactostrepcins: Bacteriocins of lactic streptococci. pp. 225-229. *In* D. Schlessinger (ed.), Microbiology-1982. American Society for Microbiology, Washington, DC.
39. Dodd, H. M., N. Horn, and M. J. Gasson. 1990. Analysis of the genetic determinant for production of the peptide antibiotic nisin. J. Gen. Microbiol. 136:555-566.
40. Donkersloot, J. A., and J. Thompson. 1990. Simultaneous loss of N^5-(Carboxyethyl)ornithine synthase, nisin production, and sucrose-fermenting ability by *Lactococcus lactis* K1. J. Bacteriol. 172:4122-4126.

41. Fleming, H. P., J. L. Etchells, and R. N. Colstilow. 1975. Microbial inhibition by an isolate of *Pediococcus* from cucumber brines. Appl. Microbiol. 30:1040-1042.
42. Foegeding, P. M., A. B. Thomas, D. H. Pilkington, and T. R. Klaenhammer. 1992. Enhanced control of *Listeria monocytogenes* by in situ-produced pediocin during dry fermented sausage production. Appl. Environ. Microbiol. 58:884-890.
43. Food and Drug Administration. 1988. Nisin preparation: affirmation of GRAS status as a direct human food ingredient. Fed. Regist. 53:11247.
44. Froseth, B. R., R. E. Herman, and L. L. McKay. 1988. Cloning of nisin resistance determinant and replication origin on 7.6-kilobase EcoRI fragment of pNP40 from *Streptococcus lactis* subsp. *diacetylactis* DRC3. Appl. Environ. Microbiol. 54:2136-2139.
45. Fuchs, P. G., J. Zajdel, and W. T. Dobrzanski. 1975. Possible plasmid nature of the determinant for production of the antibiotic nisin in some strains of *Streptococcus lactis*. J. Gen. Microbiol. 88:189-192.
46. Gao, F. H., T. Abee, and W. N. Konings. 1991. Mechanism of action of the peptide antibiotic nisin in liposomes and cytochrome C oxidase-containing proteoliposomes. Appl. Environ. Microbiol. 57:2164-2170.
47. Geis, A. 1989. Antagonistic compounds produced by lactic acid bacteria. Kiel. Milchwirtsch. Forschungsber. 41:97-104.
48. Geis, A., J. Singh, and M. Teuber. 1983. Potential of lactic streptococci to produce bacteriocin. Appl. Environ. Microbiol. 45:205-211.
49. Gilliand, S. E. 1990. Health and nutritional benefits from lactic acid bacteria. FEMS Microbiol. Rev. 87:175-178.
50. Gireesh, T., B. E. Davidson, and A. J. Hillier. 1992. Conjugal transfer in *Lactococcus lactis* of a 68-kilobase-pair chromosomal fragment containing the structural gene for the peptide bacteriocin nisin. Appl. Environ. Microbiol. 58:1670-1676.
51. Gonzalez, C. F., and B. S. Kunka. 1987. Plasmid-associated bacteriocin production and sucrose fermentation in *Pediococcus acidilactici*. Appl. Environ. Microbiol. 53:2534-2538.
52. Graham, D. C., and L. L. McKay. 1985. Plasmid DNA in strains of *Pediococcus cerevesiae* and *Pediococcus pentosaceus*. Appl. Environ. Microbiol. 50:532-534.
53. Gross, E., and J. L. Morell. 1967. The presence of dehydroalanine in the antibiotic nisin and its relationship to activity. J. Am. Chem. Soc. 89:2791-2798.
54. Gross, E., and J. L. Morell. 1970. Nisin. The assignment of sulfide bridges of β-methyllanthionine to a novel bicyclic structure of identical ring size. J. Am. Chem. Soc. 92:2919-2925.
55. Hansen, J. N., S. Banerjee, and G. W. Buchman. 1990. Structure, organization, expression, and evolution of the genes encoding the peptide precursors of nisin and subtilin. pp. 75-89. *In* D. D. Bills and S. Kung (ed.), Biotechnology and food safety. Butterworth-Heinemann, Boston.
56. Harding, C. D., and B. G. Shaw. 1990. Antimicrobial activity of *Leuconostoc gelidum* against closely related species and *Listeria monocytogenes*. J. Appl. Bacteriol. 69:648-654.
57. Hardy, K. G. 1982. Microbe-microbe interactions, bacteriocins. pp. 368-378. *In* R. G. Burns and J. H. Slater (ed.), Experimental microbial ecology. Blackwell Scientific Publications, St. Louis.
58. Harmon, K. S., and L. L. McKay. 1987. Restriction enzyme analysis of lactose and bacteriocin plasmids from *Streptococcus lactis* subsp. *diacetylactis* WM_4 and cloning of *BclI* fragments coding for bacteriocin production. Appl. Environ. Microbiol. 53:1171-1174.
59. Harris, L. J., H. P. Fleming, and T. R. Klaenhammer. 1992. Characterization of two nisin-producing *Lactococcus lactis* subsp. *lactis* strains isolated from commercial sauerkraut fermentation. Appl. Environ. Microbiol. 58:1477-1483.
60. Harris, L. J., H. P. Fleming, T. R. Klaenhammer. 1992. Novel paired starter culture system for sauerkraut, consisting of a nisin-resistant *Leuconostoc mesenteroides* strain and a nisin-producing *Lactococcus lactis* strain. Appl. Environ. Microbiol 58:1484-1489.
61. Harris, L. J., H. P. Fleming, and T. R. Klaenhammer. 1992. Developments in nisin research. Food Res. Int. 25:57-66.
62. Hastings, J. W. 1992. Cloning and nucleotide sequence of a *Leuconostoc* bacteriocin operon, abstr. P-53, p. 332. Abstr. 92nd Annu. Meet. Am. Soc. Microbiol., 1992.
63. Hastings, J. W., M. Sailer, K. Johnson, K. L. Roy, J. C. Vederas, and M. E. Stiles. 1991. Characterization of leucocin A-UAL 187 and cloning of the bacteriocin gene from *Leuconostoc gelidum*. J. Bacteriol. 173:7491-7500.
64. Hastings, J. W., and M. E. Stiles. 1991. Antibiosis of *Leuconostoc gelidum* isolated from meat. J. Appl. Bacteriol. 70:127-134.
65. Henderson, J. T., A. L. Chopko, and P. D. van Wassenaar. 1992. Purification and primary structure of pediocin PA-1 produced by *Pediococcus acidilactici* PAC-1.0. Arch. Biochem. Biophysics 295:5-12.
66. Holo, H., O. Nilssen, and I. F. Nes. 1991. Lactococcin A, a new bacteriocin from *Lactococcus lactis* subsp. *cremoris*: isolation and characterization of the protein and its gene. J. Bacteriol. 173:3879-3887.
67. Hoover, D. G. 1992. Bacteriocins: activities and applications. pp. 181-190. *In* J. L. Lederberg (ed.), Encyclopedia of microbiology, vol. 1. Academic Press, Inc., New York.
68. Hoover, D. G., P. M. Walsh, K. M. Kolaetis, and M. M. Daly. 1988. A bacteriocin produced by *Pediococcus* species associated with a 5.5-megadalton plasmid. J. Food Prot. 51:29-31.
69. Hurst, A. 1981. Nisin. pp. 85-123. *In* D. Perlman (ed.), Advances in Applied Microbiology, vol. 27. Academic Press, Inc., New York.
70. Hurst, A. 1983. Nisin and other inhibitory substances from lactic acid bacteria. pp. 327-351. *In* A. L. Branen and P. M. Davidson (ed.), Antimicrobials in foods. Marcel Dekker, Inc., New York.
71. Jager, K., and S. Harlander. 1992. Characterization of a bacteriocin from *Pediococcus acidilactici* PC and comparison of bacteriocin-producing strains using molecular typing procedures. Appl. Microbiol. Biotechnol. 37:631-637.
72. Joerger, M. C., and T. R. Klaenhammer. 1986. Characterization and purification of helveticin J and evidence for a chromosomally determined bacteriocin produced by *Lactobacillus helveticus* 481. J. Bacteriol. 167:439-446.
73. Joerger, M. C., and T. R. Klaenhammer. 1990. Cloning, expression, and nucleotide sequence of the *Lactobacillus helveticus* 481 gene encoding the bacteriocin helveticin J. J. Bacteriol. 172:6339-6347.
74. Kaletta, C., and K.-D. Entian. 1989. Nisin, a peptide antibiotic: cloning and sequencing of the *nis*A gene and posttranslational processing of its peptide product. J. Bacteriol. 171:1597-1601.
75. Kim, W. J., B. Ray, and M. C. Johnson. 1992. Plasmid transfers by conjugation and electroporation in *Pediococcus acidilactici*. J. Appl. Bacteriol. 72:201-207.
76. Klaenhammer, T. R. 1988. Bacteriocins of lactic acid bacteria. Biochimie. 70:337-349.
77. Konisky, J. 1978. The bacteriocins. pp 71-136. *In* I. C. Gunsalus, L. N. Ornston, and J. R. Sokatch (ed.), The bacteria, a treatise on structure and function. vol. 6. Bacterial diversity. Academic Press, Inc., New York.
78. Kordel, M., and H.-G. Sahl. 1986. Susceptibility of bacterial, eukaryotic, and artificial membranes to the disruptive action of the cationic peptides pep 5 and nisin. FEMS Microbiol. Lett. 34:139-144.
79. Kordel, M., F. Schuller, and H.-G. Sahl. 1989. Interaction of the pore forming-peptide antibiotics pep 5, nisin, and subtilin with non-energized liposomes. FEBS Lett. 44:99-102.
80. Kozak, W., J. Bardwoski, and W. T. Dobrzanski. 1978. Lactostrepcins-acid bacteriocins produced by lactic streptococci. J. Dairy Res. 45:247-257.
81. Lewus, C. B., A. Kaiser, and T. J. Montville. 1991. Inhibition of food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl. Environ. Microbiol. 57:1683-1688.
82. Lewus, C. B., and T. J. Montville. 1991. Detection of bacteriocins produced by lactic acid bacteria. J. Microbiol. Meth. 13:145-150.
83. Lewus, C. B., and T. J. Montville. 1992. Further characterization of bacteriocins plantaricin BN, bavaricin MN and pediocin A. Food Biotechnol. 6:153-174.
84. Lewus, C. B., S. Sun, and T. J. Montville. 1992. Production of an amylase-sensitive bacteriocin by an atypical *Leuconostoc paramesenteroides* strain. Appl. Environ. Microbiol. 58:143-149.
85. Lindgren, S. W., and W. J. Dobrogosz. 1990. Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS Microbiol. Rev. 87:149-164.
86. Lipinska, E. 1976. Nisin and its applications. pp. 103-130. *In* M. Woodbine (ed.), Antibiotics and antibiosis in agriculture. Butterworths, London.
87. Liu, W., and J. N. Hansen. 1990. Some chemical and physical properties of nisin, a small-protein antibiotic produced by *Lactococcus lactis*. Appl. Environ. Microbiol. 56:2551-2558.
88. Lozano, J. C. N., J. N. Meyer, K. Sletten, C. Pelaz, and I. F. Nes. 1992. Purification and amino acid sequence of a bacteriocin produced

by *Pediococcus acidilactici*. J. Gen. Microbiol. 138:1985-1990.
89. Luchansky, J. B., K. A. Glass, K. D. Harsono, A. J. Degnan, N. G. Faith, B. Cauvin, G. Baccus-Taylor, K. Arihara, B. Bater, A. J. Maurer, and R. G. Cassens. 1992. Genomic analysis of *Pediococcus* starter cultures used to control *Listeria monocytogenes* in turkey summer sausage. Appl. Environ. Microbiol. 58:3053-3059.
90. Maisner-Patin, S., N. Deschamps, S. R. Tatini, and J. Richard. 1992. Inhibition of *Listeria monocytogenes* in Camembert cheese made with a nisin-producing starter. Lait 72:249-263.
91. Marugg, J. D., C. F. Gonzalez, B. S. Kunka, A. M. Ledeboer, M. J. Pucci, M. Y. Toonen, S. A. Walker, L. C. M. Zoetmulder, and P. A. Vandenbergh. 1992. Cloning, expression, and nucleotide sequence of genes involved in production of pediocin PA-1, a bacteriocin from *Pediococcus acidilactici* PAC1.0. Appl. Environ. Microbiol. 58:2360-2367.
92. Mayr-Harting, A., A. J. Hedges, and R. C. W. Berkeley. 1972. Methods for studying bacteriocins. pp. 315-422. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 7a. Academic Press, Inc., New York.
93. Mortvedt, C. I., and I. F. Nes. 1990. Plasmid-associated bacteriocin production by a *Lactobacillus sake* strain. J. Gen. Microbiol. 136:1601-1607.
94. Mortvedt, C. I., J. Nissen-Meyer, K. Sletten, and I. F. Nes. 1991. Purification and amino acid sequence of lactocin S, a bacteriocin produced by *Lactobacillus sake* L45. Appl. Environ. Microbiol. 57: 1829-1834.
95. Motlagh, A. M., A. K. Bhunia, F. Szostek, T. R. Hansen, M. C. Johnson, and B. Ray. 1992. Nucleotide and amino acid sequence of *pap*-gene (pediocin AcH) production in *Pedicoccus acidilactici* H. Lett. Appl. Microbiol. 15:45-48.
96. Muriana, P. M., and T. R. Klaenhammer. 1987. Conjugal transfer of plasmid-encoded determinants for bacteriocin production and immunity in *Lactobacillus acidophilus* 88. Appl. Environ. Microbiol. 53: 553-560.
97. Muriana, P. M., and T. R. Klaenhammer. 1991. Purification and partial characterization of lactacin F, a bacteriocin produced by *Lactobacillus acidophilus* 11088. Appl. Environ. Microbiol. 57:114-121.
98. Muriana, P. M., and T. R. Klaenhammer. 1991. Cloning, phenotypic expression, and DNA sequence of the gene for lactacin F, an antimicrobial peptide produced by *Lactobacillus* spp. J. Bacteriol. 173:1779-1788.
99. Nettles, C. G. 1992. Purification and amino acid composition of the *Lactobacillus acidophilus* bacteriocin, lactacin B. Ph.D. dissertation. Clemson University, Clemson, SC.
100. Neve, J., A. Geis, and M. Teuber. 1984. Conjugal transfer and characterization of bacteriocin plasmids in group N (lactic acid) streptococci. J. Bacteriol. 157:833-838.
101. Nielsen, J. W., J. S. Dickson, and J. D. Crouse. 1990. Use of bacteriocin produced by *Pediococcus acidilactici* to inhibit *Listeria monocytogenes* associated with fresh meat. Appl. Environ. Microbiol. 56:2142-2145.
102. Ogden, K., and R. S. Tubb. 1985. Inhibition of beer-spoilage lactic acid bacteria by nisin. J. Inst. Brew. 91:390-392.
103. Orberg, P. K., and W. E. Sandine. 1984. Common occurrence of plasmid DNA and vancomycin resistance in *Leuconostoc* spp. Appl. Environ. Microbiol. 48:1129-1133.
104. Oxford, A. E., 1944. Diplococcin, an anti-bacterial protein elaborated by certain milk streptococci. Biochem. J. 38:178-182.
105. Parente, E., and C. Hill. 1992. A comparison of factors affecting the production of two bacteriocins from lactic acid bacteria. J. Appl. Bacteriol. 73:290-298.
106. Piard, J.-C., F. Delorme, G. Giraffa, J Commissaire, and M. Desmazeaud. 1990. Evidence for a bacteriocin produced by *Lactococcus lactis* CNRZ 481. Neth. Milk Dairy J. 44:143-158.
107. Piard, J.-C., and M. J. Desmazeaud. 1992. Inhibiting factors produced by lactic acid bacteria. 2. Bacteriocins and other antibacterial substances. Lait 72:113-142.
108. Piard, J.-C., P. M. Muriana, M. J. Desmazeaud, and T. R. Klaenhammer. 1992. Purification and partial characterization of lacticin 481, a lanthionine-containing bacteriocin produced by *Lactococcus lactis* subsp. *lactis* CNRZ481. Appl. Environ. Microbiol. 58:279-284.
109. Pucci, J. J., E. R. Vedamuthu, B. S. Kunka, and P. A. Vandenbergh. 1988. Inhibition of *Listeria monocytogenes* by using bacteriocin PA-1 produced by *Pediococcus acidilactici* PAC 1.0. Appl. Environ. Microbiol. 54:2349-2353.
110. Quadri, L., M. Sailer, K. Roy, J. C. Vederas, and M. E. Stiles. 1992. Characterization and genetic determinants of bacteriocins produced by *Carnobacterium piscicola* LV17B, abstr. P-57, p. 333. Abstr. 92nd Annu. Meet. Am. Soc. Microbiol. 1992.
111. Rammelsberg, M., E. Muller, and F. Radler. 1990. Caseicin 80: purification and characterization of a new bacteriocin from *Lactobacillus casei*. Arch. Microbiol. 154:249-252.
112. Rammelsberg, M., and F. Radler. 1990. Antibacterial polypeptides of *Lactobacillus* species. J. Appl. Bacteriol. 69:177-184.
113. Ray, B., A. Motlagh, M. C. Johnson, and F. Bozoglu. 1992. Mapping of pSMB74, a plasmid-encoding bacteriocin, pediocin AcH, production (Pap$^+$) by *Pediococcus acidilactici* H. Lett. Appl. Microbiol. 15:35-37.
114. Ray, S., M. C. Johnson, and B. Ray. 1989. Bacteriocin plasmids of *Pediococcus acidilactici*. J. Indust. Microbiol. 4:163-171.
115. Ray, S. K., W. J. Kin, M. C. Johnson, and B. Ray. 1989. Conjugal transfer of a plasmid encoding bacteriocin production and immunity in *Pediococcus acidilactici* H. J. Appl. Bacteriol. 66:393-399.
116. Rayman, K., and A. Hurst. 1984. Nisin properties, biosynthesis, and fermentation. pp. 607-628. *In* E. J. Vandamme (ed.), Biotechnology of Industrial Antibiotics. Marcel Dekker, Inc., New York.
117. Reeves, P. 1965. The bacteriocins. Bacteriol. Rev. 29:24-45.
118. Ruechert, P. W. 1979. Studies on a bacteriocin-like activity produced by *Pediococcus acidilactici* effective against gram positive organisms. M.S. thesis. Michigan State University, East Lansing.
119. Ruhr, E., and H. G. Sahl. 1985. Mode of action of the peptide antibiotic nisin and influence on the membrane potential of whole cells and cytoplasmic and artificial membrane vesicles. Antimicrob. Agents Chemother. 27:841-845.
120. Sandine, W. E. 1979. Roles of lactobacillus in the intestinal tract. J. Food Prot. 42:259-262.
121. Sandine, W. E. 1990. Roles of bifidobacteria and lactobacilli in human health. Contemp. Nutr., vol. 15.
122. Scherwitz, K. M., K. A. Baldwin, and L. L. McKay. 1983. Plasmid linkage of a bacteriocin-like substance in *Streptococcus lactis* subsp. *diacetylactis* strain WM_4: transferability to *Streptococcus lactis*. Appl. Environ. Microbiol. 45:1506-1512.
123. Schillinger, U. 1990. Bacteriocins of lactic acid bacteria. pp. 55-74. *In* D. D. Bills and S. Kung (ed.), Biotechnology and food safety. Butterworth-Heinemann, Boston.
124. Schillinger, U., and W. H. Holzapfel. 1990. Antibacterial activity of carnobacteria. Food Microbiol. 7:305-310.
125. Schillinger, U., M. Kaya, and F.-K. Lucke. 1991. Behaviour of *Listeria monocytogenes* in meat and its control by bacteriocin-producing strain of *Lactobacillus sake*. J . Appl. Bacteriol. 70:473-478.
126. Schillinger, U., and F.-K., Lucke. 1989. Antibacterial activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Microbiol. 55:1901-1906.
127. Sobrino, O. J., J. M. Rodriguez, W. L Moreira, M. F. Fernandez, B. Sanz, and P. E. Hernandez. 1991. Antibacterial activity of *Lactobacillus sake* isolated from dry fermented sausages. Int. J. Food Microbiol. 13:1-10.
128. Sobrino, O. J., J. M. Rodriguez, W. L. Moreira, M. F. Fernandez, B. Sanz, and P. E. Hernandez. 1992. Sakacin M, a bacteriocin-like substance from *Lactobacillus sake* 148. Int. J. Food Microbiol. 16:215-225.
129. Speck, M. L. 1976. Interactions among lactobacilli and man. J. Dairy Sci. 59:338-343.
130. Speck, M. L. 1980. Preparation of lactobacilli for dietary uses. J. Food Prot. 42:65-67.
131. Spelhaug, S. R., and S. K. Harlander. 1989. Inhibition of foodborne bacterial pathogens by bacteriocins from *Lactococcus lactis* and *Pediococcus pentosaceous*. J. Food Prot. 52:856-862.
132. Steele, J. L., and L. L. McKay. 1986. Partial characterization of the genetic basis for sucrose metabolism and nisin production in *Streptococcus lactis*. Appl. Environ. Microbiol. 51: 57-61.
133. Steen, M. T., Y. J. Chung, and J. N. Hansen. 1991. Characterization of nisin gene as part of a polycistronic operon in the chromosome of *Lactococcus lactis* ATCC 11454. Appl. Environ. Microbiol. 57:1181-1188.
134. Stevens, K. A., N. A. Klapes, B. W. Sheldon, and T. R. Klaenhammer. 1992. Antimicrobial action of nisin against *Salmonella typhimurium* lipopolysaccharide mutants. Appl. Environ. Microbiol. 58:1786-1788.

135. Stevens, K. A., B. W. Sheldon, N. A. Klapes, and T. R. Klaenhammer. 1991. Nisin treatment for inactivation of *Salmonella* species and other gram negative bacteria. Appl. Environ. Microbiol. 57:3613-3615.
136. Stevens, K. A., B. W. Sheldon, N. A. Klapes, and T. R. Klaenhammer. 1992. Effect of treatment conditions on nisin inactivation of gram-negative bacteria. J. Food Prot. 55:763-766.
137. Stoddard, G. W., J. P. Petzel, M. J. van Belkum, J. Kok, and L. L. McKay. 1992. Molecular analyses of the lactococcin A gene cluster from *Lactococcus lactis* ssp. *lactis* biovar *diacetylactis* WM_4. Appl. Environ. Microbiol. 58:1952-1961.
138. Stoffels, G., I. F. Nes, and A. Gudmundsdottir. 1992. Isolation and properties of a bacteriocin-producing *Carnobacterium piscicola* isolated from fish. J. Appl. Bacteriol. 73:309-316.
139. Stoffels, G., J. Nissen-Meyer, A. Gudmundsdottir, K. Sletten, H. Holo, and I. F. Nes. 1992. Purification and characterization of a new bacteriocin isolated from *Carnobacterium* sp. Appl. Environ. Microbiol. 58:1417-1422.
140. Tagg, J. R., A. S. Dajani, and L. W. Wannamaker. 1976. Bacteriocins of gram positive bacteria. Bacteriol. Rev. 40:722-756.
141. Tichaczek, P. S., J. N. Meyer, I. F. Nes, R. F. Vogel, and W. P. Hammes. 1992. Characterization of the bacteriocins curvacin A from *Lactobacillus curvatus* LTH1174 and sakacin P from *L. sake* LTH673. System. Appl. Microbiol. 15:460-468.
142. Tsai , H. -J ., and W. E. Sandine. 1987. Conjugal transfer of nisin plasmid genes from *Streptococcus lactis* 7962 to *Leuconostoc dextranicum*. Appl. Environ. Microbiol. 53:352-357.
143. Upreti, G. C., and R. D. Hinsdill. 1973. Isolation and characterization of a bacteriocin from homofermentative *Lactobacillus*. Antimicrob. Agents Chemother. 4:487-494.
144. Upreti, G. C., and R. D. Hinsdill. 1975. Production and mode of action of lactocin 27:bacteriocin from a homofermentative *Lactobacillus*. Antimicrob. Agents Chemother. 7:139-145.
145. van Belkum, M. J., B. J. Hayema, A. Geis, J. Kok, and G. Venema. 1989. Cloning of two bacteriocin genes from a lactococcal bacteriocin plasmid. Appl. Environ. Microbiol. 5:1187-1191.
146. van Belkum, M. J., B. J. Hayema, R. E. Jeeninga, J. Kok, and G. Venema. 1991. Organization and nucleotide sequences of two lactococcal bacteriocin operons. Appl. Environ. Microbiol. 57:492-498.
147. van Belkum, M. J., J. Kok, and G. Venema. 1992. Cloning, sequencing, and expression in *Escherichia coli* of *lcn*B, a third bacteriocin determinant from the lactococcal bacteriocin plasmid p9B4-6. Appl. Environ. Microbiol. 53:572-577.
148. van Laack, R. L. J. M., U. Schillinger, and W. H. Holzapfel. 1992. Characterization and partial purification of a bacteriocin produced by *Leuconostoc carnosum* LA44A. Int. J. Food Microbiol. 16:183-195.
149. Vaughan, E. E., C. Daly, and G. F. Fitzgerald. 1992. Identification and characterization of helveticin V-1829, a bacteriocin produced by *Lactobacillus helveticus* 1829. J. Appl. Bacteriol. 73:299-308.
150. West, C. A., and P. J. Warner. 1988. Plantacin B, a bacteriocin produced by *Lactobacillus plantarum* NCDO 1193. FEMS Microbiol. Lett. 49:163-165.
151. Worobo, R., T. Henkel., K. Roy, J. C. Vederas, and M. E. Stiles. 1992. Characterization and genetic determinants of carnobacteriocin isolated from *Carnobacterium piscicola* LV17A., abstr. P-56, p. 333. Abstr. 92nd Annu. Meet. Am. Soc. Microbiol. 1992.
152. Zajdel, J. K., P. Ceglowski, and W. T. Dobrzanski. 1985. Mechanism of action of lactostrepcin 5, a bacteriocin produced by *Streptococcus cremoris* 202. Appl. Environ. Microbiol. 49:969-974.
153. Zajdel, J. K., and W. T. Dobrzanski. 1983. Isolation and preliminary characterization of *Streptococcus cremoris* (strain 202) bacteriocin. Acta. Microbiol. Polonica 32:119-129.